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TWENTIETH CENTURY PRACTICE

AN INTERNATIONAL ENCYCLOPEDIA

OF

MODERN MEDICAL SCIENCE

BY

LEADING AUTHORITIES OF EUROPE AND AMERICA

EDITED BY

THOMAS L. STEDMAN, M.D.

NEW YORK CITY

IN TWENTY VOLUMES

VOLUME XIX

MALARIA AND MICROORGANISMS

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PLATES ILLUSTRATING

MALARIA.

BY

ETTORE MARCHIAFAVA AND AMICO BIGNAMI.

PLATE I.

FIGS. 1–14.—Hæmatozoa of Quartan Fever: Figs. 1–9, Progressive endoglobular development of the quartan parasite; Figs. 10–11, Endoglobular fission forms, Fig. 12, Free sporulation; Figs. 13–14, Free pigmented forms, one flagellated.

FIGS. 15–33.—Hæmatozoa of Tertian Fever: Figs. 15–24, Progressive endoglobular development of the tertian parasite; Figs. 25–27, Endoglobular fission forms; Figs. 28–30, Free sporulations; Figs. 31–33, Free pigmented forms, one flagellated.

FIGS. 34–55.—Hæmatozoa of Estivoautumnal (Quotidian) Fever: Figs. 34–50, Endoglobular development of the quotidian parasite; Figs. 42, 48, and 49, Parasites in altered red blood corpuscles (brassy bodies); Figs. 51–55, Endoglobular forms in sporulation.

All the figures of this plate, as well as those of the following plate, were drawn from fresh specimens ($\frac{1}{12}$ Leitz immersion); Figs. 50–55 only were drawn from a preparation of the bone marrow in a case of pernicious quotidian; the marrow was dried after Ehrlich's method on a cover-glass, and stained with methylene blue.

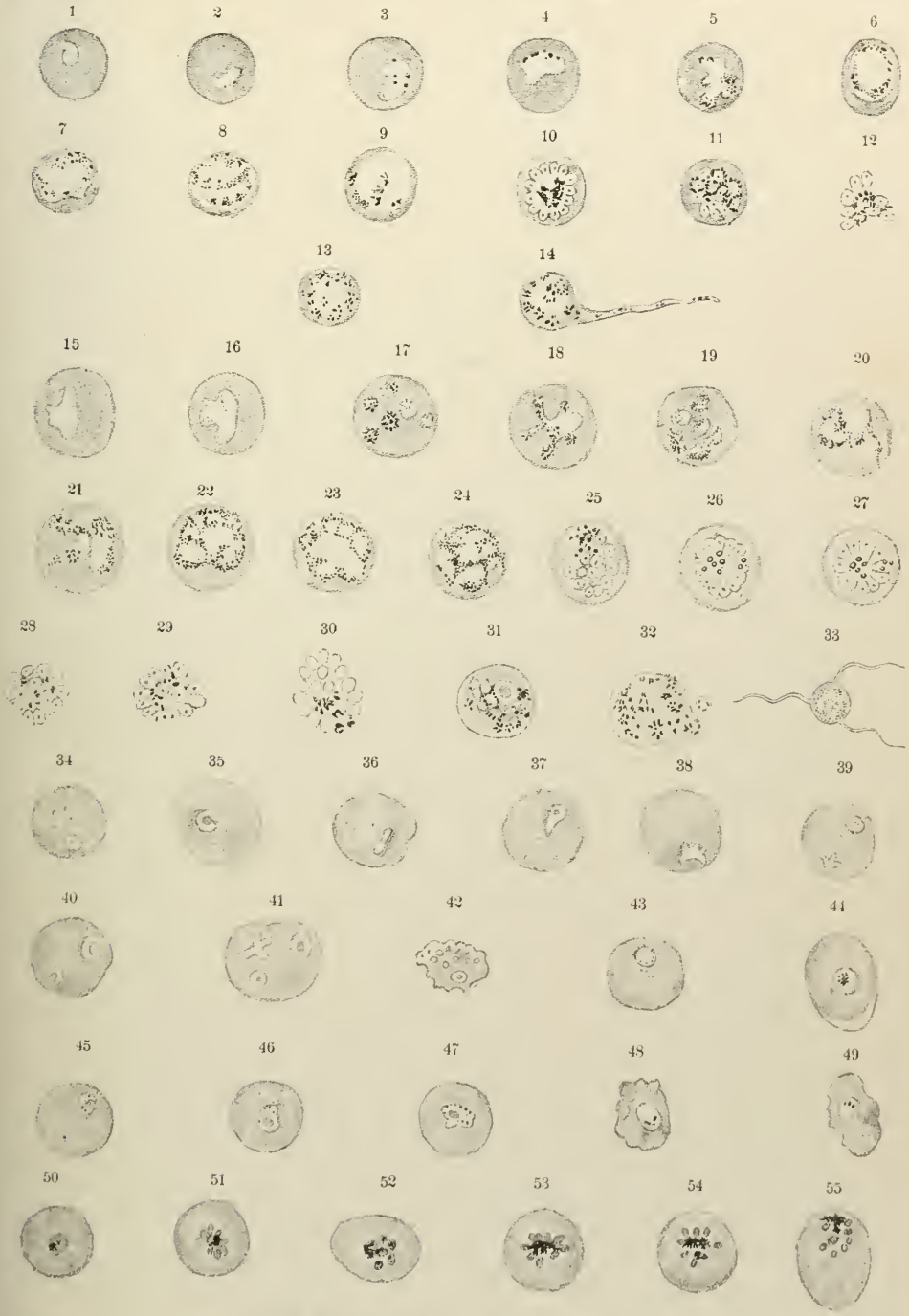

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PLATE II.

FIGS. 1–48.—Hæmatozoa of Estivoautumnal (Tertian) Fever: Figs. 1–9, Young non-pigmented parasites; Figs. 10–32, Parasites in process of development, pigmented at the periphery; Figs. 33–42, Parasites very near the stage of multiplication, the pigment in which is collected near the centre; Figs. 43–48, Sporulation forms. In Figs. 8 and 9, and 30–42 are represented the various changes occurring in the red blood corpuscles invaded by the parasites.

FIGS. 49–70.—Various Forms Belonging to the Group of Crescent Bodies: Figs. 49–60, Fusate, ovoid, round, and semilunar endoglobular forms; Figs. 61–64, The same forms in a free state; Figs. 65–66, Crescent bodies properly so called; Figs. 67–69, Free ovoid and round bodies; Fig. 70, Vacuolization of a free crescent body.

FIGS. 71–77.—Flagellated Bodies ($\frac{1}{12}$ Leitz immersion).

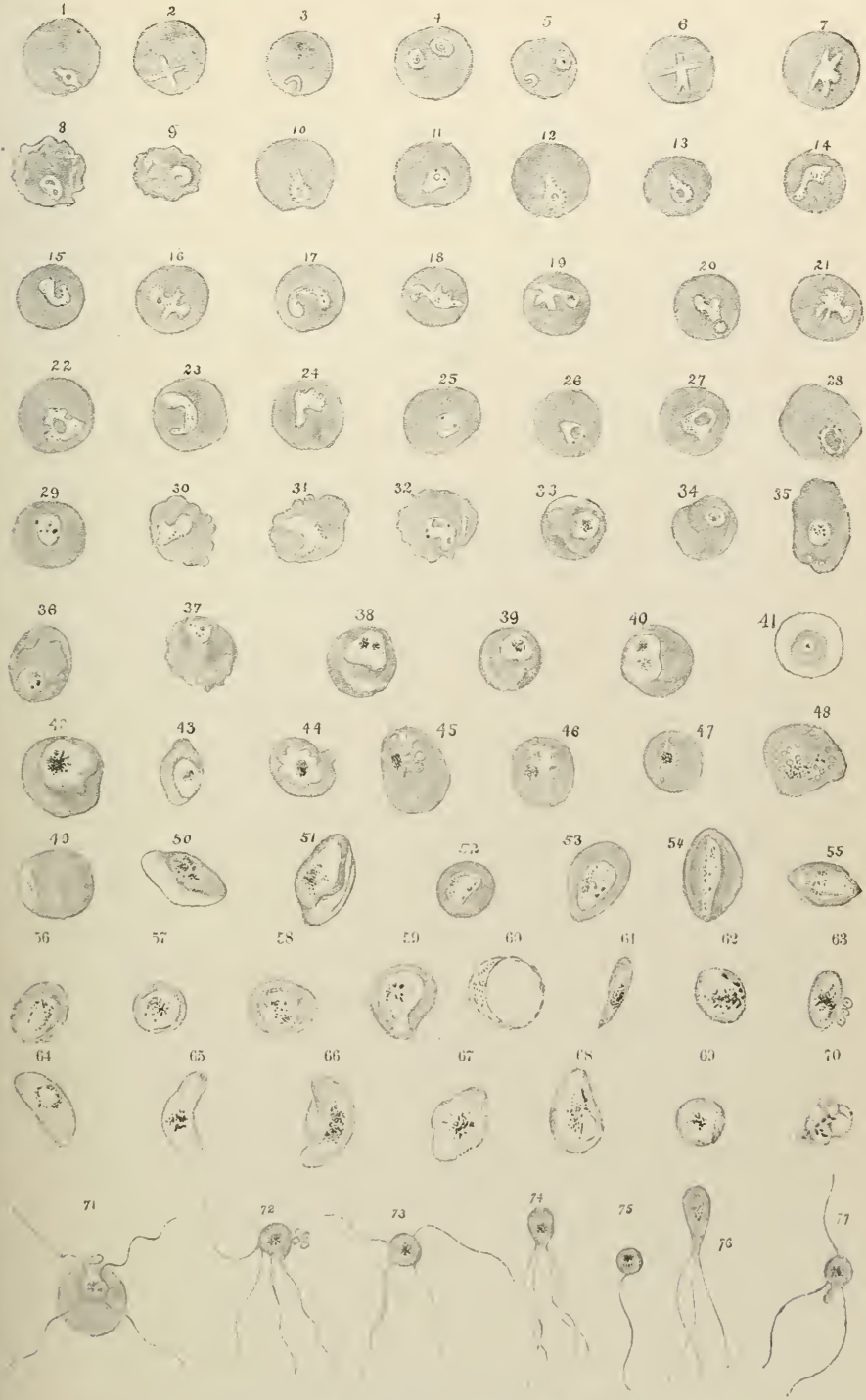

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PLATE III.

FIGS. 1–3.—Non-pigmented Parasites.

FIGS. 4–5.—Parasites Containing Pigment Granules.

FIGS. 6–12.—Parasites with Small Blocks of Pigment, in various stages of development up to fission.

FIG. 13.—A Vacuolized Parasite Containing a Small Mass of Pigment.

FIG. 14.—A Large Parasite with Granules, containing two chromatin bodies.

FIGS. 15–17.—Large Parasites with Blocks of Pigment, some not yet differentiated, others at the beginning of differentiation.

FIGS. 18–22.—Various Phases of Division.

FIG. 23.—A Leucocyte with Polymorphous Nucleus, containing spores.

FIGS. 24–25.—Spores Contained in, or Adherent to Red Corpuscles.

FIG. 26.—Division of a Parasite, with a Block of Pigment, into Irregular Masses (Disaggregation).

FIG. 27.—Division of a Parasite with a Block of Pigment into Rings, each of which contains a granule of chromatin.

FIGS. 28–30.—Red Blood Corpuscles Containing More than One Parasite, in the same or different stages of development.

FIGS. 31–33.—Small Endoglobular Crescent Bodies.

FIGS. 34–35.—Bodies with Central Pigment, which are free in the blood plasma and in a condition of vacuolization.

FIGS. 36–37.—Large Pale Pigmented Bodies of doubtful interpretation.

The figures are made from preparations of blood fixed in equal parts of alcohol and ether and stained with Ehrlich's hæmatoxylin and eosin ($\frac{1}{12}$ Zeiss homogeneous immersion lens). From Bastianelli e Bignami: "Studi sella infezione malarica." Bullettino della R. Accademia Medica di Roma, Anno XX.

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PLATE IV.

Malarial Parasites Stained by Romanowsky's Method, in which there is a selective stain of the nuclear chromatin. (From researches, as yet unpublished, of Bastianelli and Bignami.)

Figs. 1–9.—Quartan Parasites: Figs. 4–7, Various phases of nuclear division up to complete fission (Fig. 6); Fig. 8, Quartan parasite completely divided into young parasites distinctly separated one from the other, each of them having a nucleus rich in chromatin; Fig. 9, Adult quartan form, sterile in man.*

Figs. 10–22.—Tertian Parasites: Figs. 10–13, Various phases of development of the tertian parasite. Figs. 14–17, Various phases of nuclear division; Fig. 18, Complete fission of a parasite into numerous daughter corpuscles (the "sporulation" of many writers); Figs. 19–21, Adult forms of the tertian parasite, sterile in man,* from one of which (Fig. 21) a gemmule is extruding; Fig. 22, A flagellated body, derived from one of the forms above pictured, which has two flagella formed in great part of chromatin and one of protoplasm only. In the greater number of flagellated bodies all the flagella contain chromatin, but more figures are not given from want of space.

Figs. 23–45.—Estivoautumnal Parasites: Figs. 23–24, Young parasites; in Fig. 24 the parasite looks as if it were resting in a depression in the red blood corpuscle and had not wholly penetrated into its interior; Figs. 25–26, Adult parasitic forms, with pigment collected into one or more blocks, which are very near the stage of nuclear division; Figs. 27–34, Various phases of nuclear division up to complete fission of the parasite into daughter corpuscles (sporulation); Figs. 35–43, Forms of the crescent phase in various degrees of development (sterile forms in man*); Figs. 35–38, Young crescents (ovoid and fused bodies); Fig. 38, Ovoid body with a gemmule composed of protoplasm; Figs. 39 and 42, Ovoid and round bodies with gemmules composed of chromatin; Fig. 40, Swollen crescent form from which the chromatin has in large part escaped (preparation kept in the moist chamber for about a quarter of an hour before fixation); Fig. 43, Round body issuing from a crescent the chromatin of which is escaping in the form of a filament (preparation kept in the moist chamber, as above); Figs. 44–45, Flagellated bodies derived from crescent forms; in one (Fig. 44) the flagella are composed of chromatin in great part, in the other (Fig. 45) they consist of protoplasm.

* Those forms designated as sterile in man are capable of development ulteriorly in a life cycle as sporozoa in the middle intestine of Anopheles claviger (A. maculipennis, of Meigen).

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PLATE V.

FIGS. 1–10.—Development of the Crescents in the Middle Intestine of Anopheles Claviger: Fig. 1, Crescent in the wall of the middle intestine a little less than two days (about forty hours) after the Anopheles had sucked the blood of a sufferer from malaria. The parasite preserves its spindle shape, resembling perfectly the form which it may assume in the blood of man; Figs. 2–5, Forms of progressive development, surrounded by a very thin hyaline capsule, showing the phases of successive division of the nucleus. The nuclei of the parasitic body in Fig. 5 are small and very numerous; Fig. 6, Forms of complete development of the crescent sporozoon; within the capsule are seen very numerous sporozoites (Figs. 1–6 are drawn from preparations fixed in formalin and alcohol and stained with Böhmer's hæmatoxylin; Fig. 6 is from the infected intestine of Anopheles, cut *in toto* after being embedded in paraffin); Fig. 7, A mature sporozoon containing a very large number of sporozoites, seen in an unstained fresh preparation; Fig. 8, The salivary gland of Anopheles, the cells of which contain numerous sporozoites; Fig. 9, Mature sporozoites drawn from a preparation first dried, then treated with absolute alcohol, and stained by Romanowsky's method; Fig. 10, A large capsule containing many brown bodies of varied form and structure.

FIGS. 11–18.—Developmental Forms of the Parasites of Ordinary Tertian in the Middle Intestine of Anopheles Claviger: Fig. 11, Tertian body in the substance of the middle intestine less than two days after the insect had sucked the blood of a patient with tertian fever; Figs. 12–16, Later developmental forms of the tertian sporozoon, showing the successive divisions of the nucleus of the parasite; Fig. 17, Mature tertian sporozoon containing very numerous sporozoites and the residua of segmentation (semi-schematic). (All the preceding figures—11 to 17 inclusive—are drawn from preparations fixed in formalin and alcohol and stained with Böhmer's hæmatoxylin); Fig. 18, Mature tertian sporozoon containing very many sporozoites and residua of segmentation, seen in an unstained fresh preparation.

FIG. 19.—The Middle Intestine of a Specimen of Anopheles Claviger Captured in a Cabin in Ostia (a region where grave malaria prevails) occupied by several peasants suffering from malaria. It contains an enormous quantity of cystic bodies, the greater number of them mature and enclosing sporozoites. They are scattered throughout the entire length of the middle intestine, pressing toward the exterior, and are most abundant in the middle third but less abundant in the anterior third. The figure is semi-schematic, drawn after a preparation seen with a Hartnack objective 4, eyepiece 3.

FIGS. 1–10 are taken from the plates of a work about to be published by Grassi, Bignami, and Bastianelli. Figs. 11–18 are from another work soon to be published by Bastianelli and Bignami.

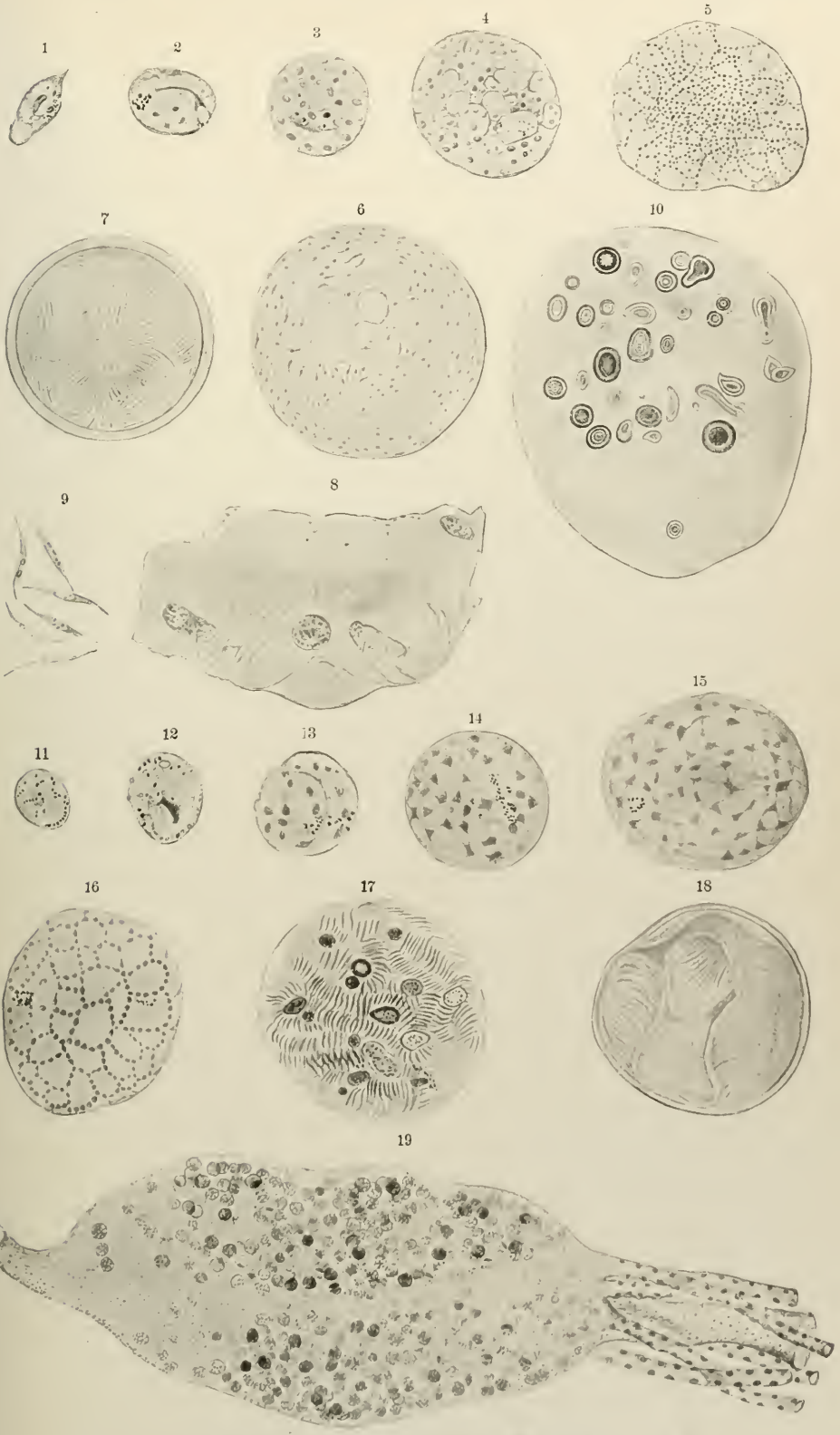

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PLATE VI.

FIGS. 1–2.—Cerebral Capillaries Containing Non-Pigmented Parasites: In Fig. 1 the parasite-containing red blood corpuscles are seen ranged along the vessel walls.

FIG. 3.—A Cerebral Capillary in which All the Red Corpuscles Contain Non-Pigmented Parasites in Various Degrees of Development; many are already in the stage of sporulation.

FIGS. 4–5.—Cerebral Capillaries Containing Endoglobular Parasites, with pigment either collected at the centre or irregularly disseminated.

FIG. 6.—Cerebral Capillary Filled with Pigmented Parasites in the Fission Stage; some of the fission forms are endoglobular, others free and in a state of disaggregation; near the fission forms are seen endoglobular bodies with pigment irregularly disseminated (*c*), others with the pigment collected into a central mass; there are also pigmented endothelial cells, and blocks of free pigment.

FIG. 7.—Cerebral Capillary Filled with Spores and Blocks of Black Pigment.

FIG. 8.—Cerebral Capillary Containing Pigmented Cells and Parasites in the Stage of Sporulation.

FIG. 9.—Bone Marrow. The medullary vessels are filled with fission forms and fusate bodies; the elements of the medullary pulp, some of which are pigmented and contain globules, are in karyokinesis. At *c* is seen a necrotic macrocyte, in which the nucleus is not recognizable, filled with decolorized red blood corpuscles containing parasites with central pigment blocks.

FIG. 10 —Macrophagi of the Bone Marrow. (*a*) Large cell in which in a large vacuole are seen parasite-containing red blood corpuscles; (*b*) macrocyte with decolorized nucleus, the nuclear membrane being preserved, containing endoglobular parasites in the fission stage, decolorized red blood corpuscles containing amœbæ, and blocks of pigment.

All these preparations were taken from cases of pernicious infection, and were stained with a saturated aqueous solution of vesuvin; seen with a $\frac{1}{12}$ Zeiss homogeneous immersion lens.

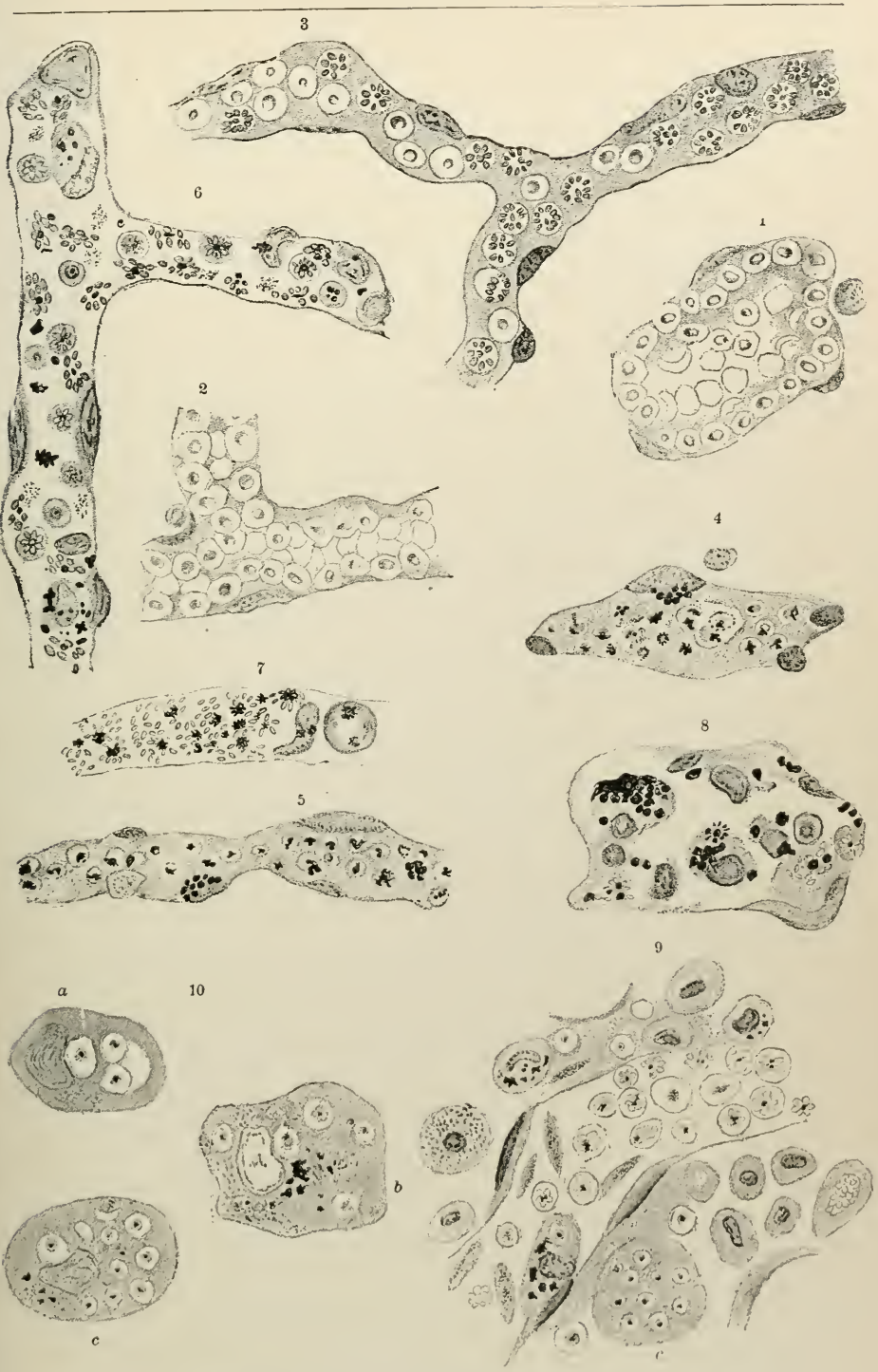

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PLATE VII.

FIG. 1.—Acute Enlargement of the Spleen in Malaria (Leitz, objective 8, ocular 3). The preparation shows most intense hyperæmia of the splenic pulp; the elements of the pulp are in part pigmented. Very many red corpuscles containing parasites with central pigment, and in process of fission, are ranged along the walls of the vasal lacunæ and interposed between the elements of the pulp.

Fig. 2.—The Liver in Pernicious Fever. The intralobular vessels contain a few endoglobular parasites, pigmented and parasite-containing macrocytes; many of the vasal endothelia are pigmented. The hepatic cells are in large part filled with blocks of yellowish, greenish-yellow, and brassy yellow pigment (Leitz, objective 6, ocular 3).

FIG. 3.—Mucosa of the Stomach from a Case of Choleraic Pernicious Fever, seen under slight magnification (Hartnack, objective 4, ocular 2). All the capillaries of the mucosa are filled with pigmented endoglobular parasites (under this enlargement only the masses of pigment are seen); the superficies of the mucosa is completely necrotic; the interglandular capillaries of the necrotic tissue as far as the superficial strata are obstructed by pigmented parasites and by melaniferous leucocytes.

FIG. 4.—Extremity of an Intestinal Villus (Zeiss, $\frac{1}{12}$ homogeneous immersion lens) from a Case of Choleraic Pernicious Fever. Some of the capillaries are filled with endoglobular pigmented parasites, some of them in the fission stage, other capillaries contain only accumulations of melaniferous leucocytes or masses of apparently free pigment. Many of the cellular elements of the villus have a nucleus stained with difficulty by the usual nuclear stains; at some points the superficies of the villus is infiltrated with large and ill-formed bacilli.

The preparations were stained with Loeffler's alcoholic solution of methylene blue.

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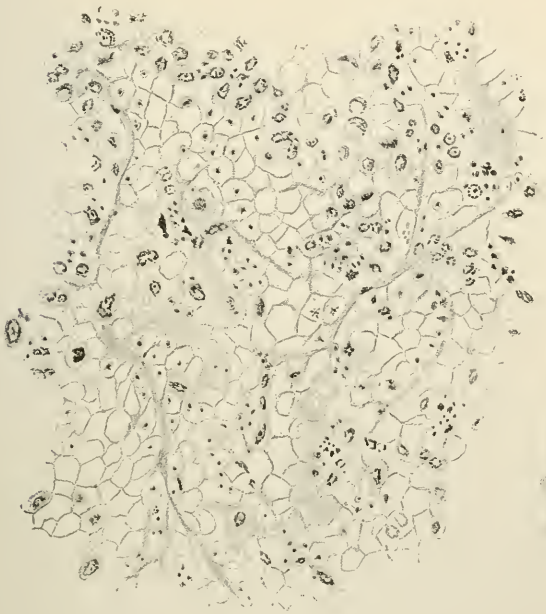

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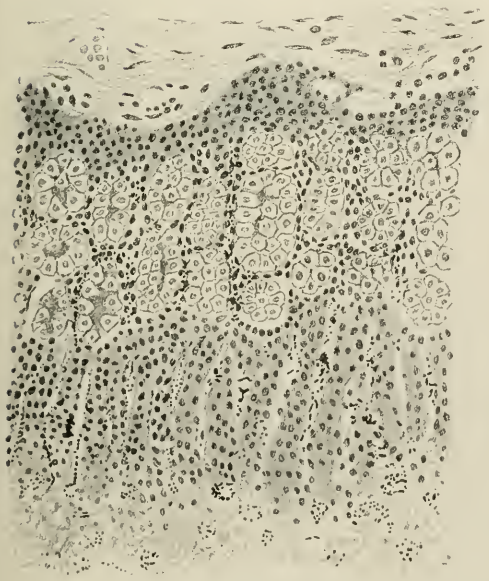

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PLATE VIII.

FIG. 1.—Section of the Brain in a Case of Comatose Pernicious Fever. The white substance is the seat of numerous punctiform hemorrhages.

FIG. 2.—Enlargement of the Liver with Perilobular Melanosis (natural size). The malarial infection had shortly before run its course.

FIG. 3.—Enlargement of the Spleen with Marked Melanosis. The follicles are enlarged and do not contain pigment.

FIG. 4.—A Pigmented Leucocyte in Process of Degeneration, in Pernicious Fever. The nucleus is not visible, the protoplasm is filled with shining droplets. Drawn from a fresh specimen and seen with $\frac{1}{12}$ Leitz homogeneous immersion.

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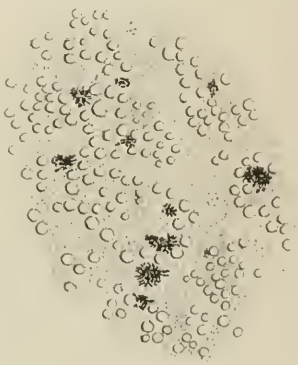


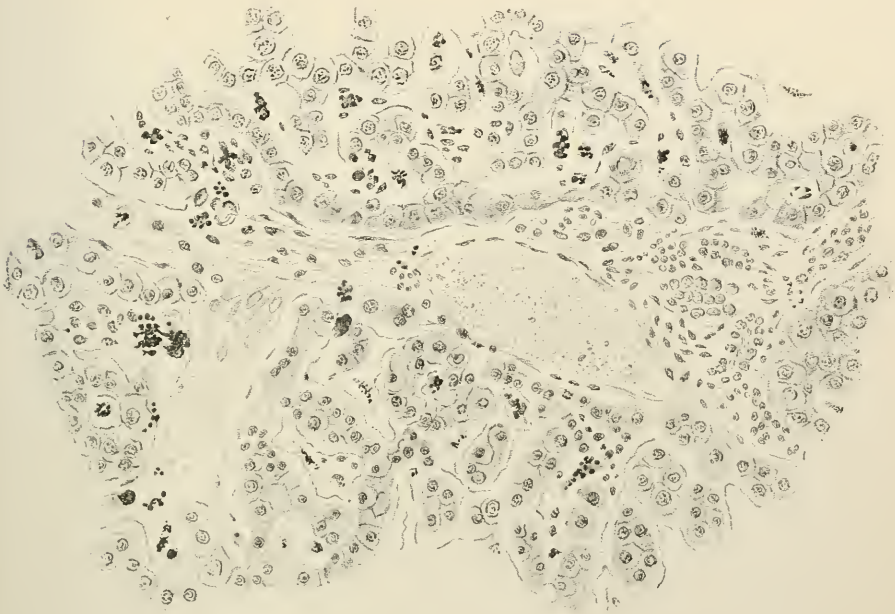
MARCHIAFAVA AND BIGNAMI—MALARIA.

PLATE IX.

FIG. 1.—Chronic Enlargement of the Liver in Malaria (Hartnack, objective 5, ocular 3). The preparation shows the melanosis of the periphery of the hepatic lobule; large pigmented elements are seen ranged along the walls of the vessels; the endothelia are pigmented; blocks of pigment are seen also in the large perivascular elements and in the lymphatics of the triangular space. Many of the hepatic cells are reduced in size, but the nucleus is preserved and of normal appearance.

FIG. 2.—Chronic Malarial Enlargement of the Liver without Melanosis (Hartnack, objective 5, ocular 3). Lymphatic dilatations and cysts; very marked atrophy of the hepatic trabeculæ interposed between the lymphatic ectasiæ; lacunar dilatation of the capillaries and stasis of the lymphatic elements in the latter.

1



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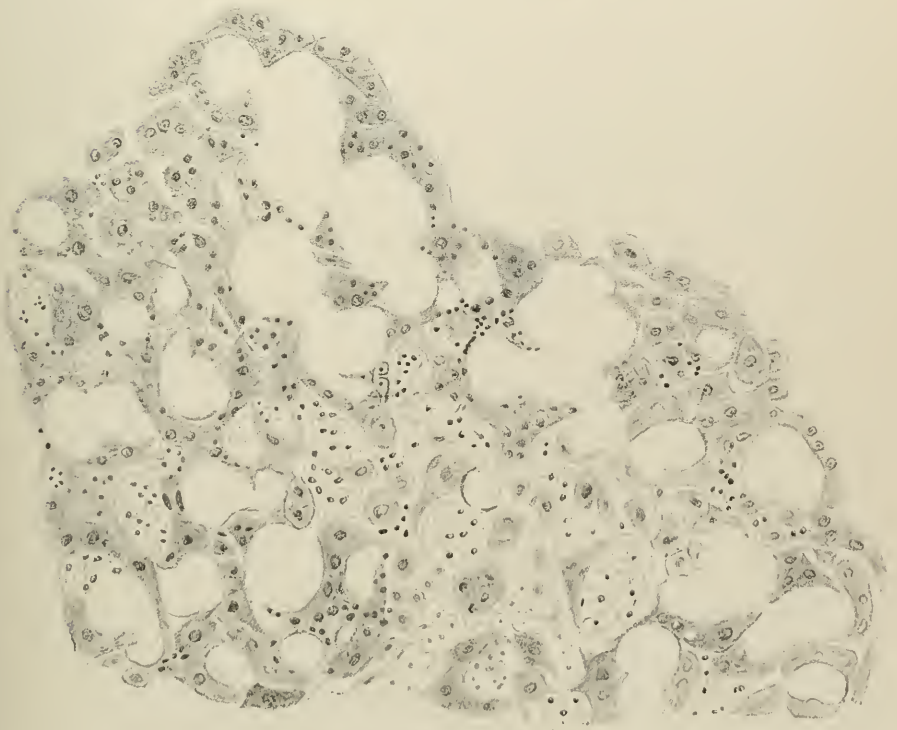

MARCHIAFAVA AND BIGNAMI—MALARIA.

PLATE X.

Fig. 1.—Chronic Enlargement of the Spleen in Malaria, and Acute Recurrent Enlargement (Hartnack, objective 5, ocular 3). Enormous dilatations of the venous lacunæ; thickening of the splenic reticulum; melanæmia and melanosis of the pulp.

Fig. 2.—Chronic Malarial Enlargement of the Spleen (Hartnack, objective 5, ocular 3). Enormous dilatations of the splenic vessels with marked atrophy and pigmentation of the splenic pulp. The splenic tissue has assumed a cavernous aspect.

Fig. 3.—Chronic Malarial Enlargement of the Spleen (Hartnack, objective 5, ocular 3). False angioma of the spleen; a large number of giant cells.

Fig. 4.—Chronic Malarial Enlargement of the Liver (Leitz, objective 6, ocular 3). The capillaries are enormously dilated, large blocks of pigment are seen along the walls of the vessels; the perivascular lymphatics are markedly dilated. The hepatic cells are atrophic and vacuolized; some of them are necrotic, others present degenerative alterations of the nucleus.

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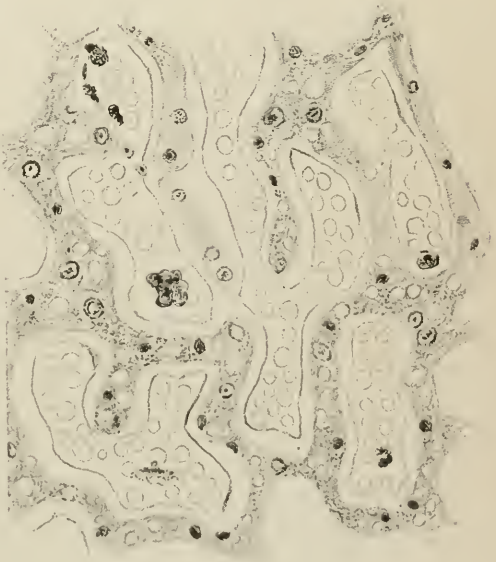


MARCHIAFAVA AND BIGNAMI—MALARIA.

PLATE XI.

The figures in this plate represent the principal alterations of the liver and the kidneys in pernicious hæmoglobinuria. The preparations from which the drawings were made were stained with hæmatoxylin and eosin.

Fig. 1.—Sections of the Liver, showing almost Complete Injection of the Biliary Capillaries by Inspissated Bile (Hartnack, objective 5, ocular 3).

Fig. 2.—Hepatic Cell in Karyokinesis Surrounded by Biliary Capillaries Filled with a Delicate Biliary Cast (Zeiss, $\frac{1}{12}$ immersion).

Figs. 3 and 4.—Hepatic Cells in Karyokinesis Containing Granules of Biliary Pigment (Zeiss, $\frac{1}{12}$ immersion).

Fig. 5.—Section of the Kidney, showing the Convoluted Tubules with Necrotic and Partly Disintegrated Epithelium (Hartnack, objective 7, ocular 3).

Fig. 6.—Renal Tubule with Exfoliated Epithelium Impregnated with Hæmoglobin (Hartnack, objection 7, ocular 3).

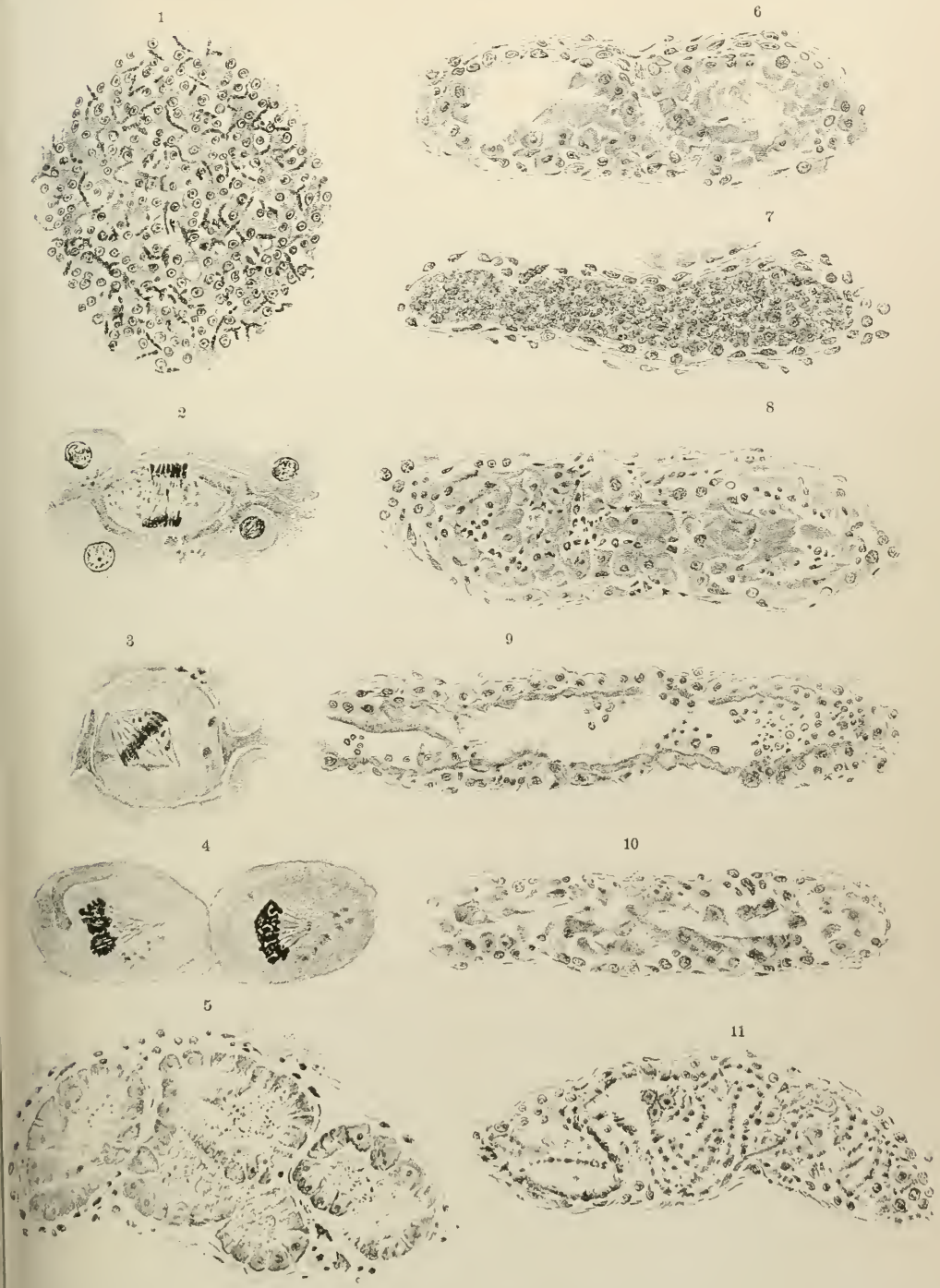
Fig. 7.—Renal Tubule Containing a Cylinder Composed of Fine Granules of Hæmoglobin; the epithelium of the tubule is not visible.

Fig. 8.—Dilated Renal Tubule Filled with Exfoliated Epithelium and with Leucocytes Impregnated with Hæmoglobin.

Fig. 9.—Renal Tubule with the Epithelium in Great Part Preserved, upon which is Deposited a Layer of Hæmoglobin; in the lumen of the tubule are seen exfoliated epithelial cells and numerous white blood corpuscles.

Fig. 10.—Renal Tubule Containing Necrotic Epithelia Impregnated with Bile Pigment.

Fig. 11.—Renal Tubule Containing a Tangle of Rosary-like Filaments of Biliary Pigment.



MARCHIAFAVA AND BIGNAMI—MALARIA.

MALARIA.

BY

ETTORE MARCHIAFAVA,

AND

AMICO BIGNAMI,

ROME.

MALARIA.

THIS article on malaria was to have been prepared and published over a year ago, but the discoveries regarding the malarial parasite made while the preparation of the article was in progress so modified our conceptions in several essential points that it was found necessary to rewrite entirely certain sections and to change others. In the course of little more than six months our knowledge of the biology of the malarial parasites outside of the human body, concerning which up to that time there were merely more or less well-founded suppositions and hypotheses, has so wonderfully increased that we may say now that it is as well known, in certain parts at least, as is the natural history of the same parasites in the blood. A question of such great practical importance for the physician as the mode of entrance of the parasites into man has been determined experimentally, and where only a few months ago doubt was reasonable and prudent now there is certainty. Certain biological phases of the malarial parasites, concerning which but a short time ago there were many discussions and much divergence of opinion, have now a well-recognized and established biological significance. On the other side, this noteworthy progress has opened up a new horizon, revealed new problems, started new discussions, and shown new fields for research in which already from many sides the work has been begun. The increase of our knowledge of the biology of the parasites has also naturally modified our views regarding the epidemiology of malaria, and has pointed out to us a new and certain method of prophylaxis.

Whoever has followed this rapid movement will not be surprised to learn that in certain works on malaria, published even during the past twelvemonth, and which are otherwise most valuable, various questions are studied in a way which now appears most incomplete; and he will readily understand the desire of the present writers to delay the final revision of their work until they had acquired an experience and a personal conviction regarding the most important questions in order to be in a position to discuss intelligently the observations and views of other workers in the same field.

In this treatise the sections on Parasitology and Etiology are those

which have been modified the most as a result of the latest researches. The sections on Pathology and Pathological Anatomy contain for the most part the results of studies made since the discovery of the malarial parasite. In the section on Symptomatology we have almost entirely omitted reference to those clinical forms which, because believed to be malarial, were treated of at length in works published before the discovery of the parasite, but which are now admitted to be due to other causes. We have devoted a special section to the subject of Tropical Malaria, to which much attention has recently been given by investigators in many tropical countries; and we have also included in our treatise a consideration of Hæmoglobinuria. In view of the recent discoveries of the manner in which malarial infection is acquired, the incompleteness of our present efforts in the way of prophylaxis will doubtless be remedied in the immediate future, and there is very much to be hoped for in that direction as a result of the great activity manifested in all parts of the world in the study of malaria.

Historical Sketch.

The history of the researches which have been made into the subject of malarial infection may be divided into three periods: the first, or ancient, period; the second, dating from the discovery of the specific remedy; and the third, or recent, period, which dates from the discovery of the parasite producing the infection.

The earliest theories in regard to intermittent fever are lost in the obscurity which reigns over the origin of medical science.

It would appear that to Empedocles of Agrigento (fifth century before Christ) we owe the first knowledge of the injurious effect of stagnant water upon the health of man. He is said to have delivered Selinunte from an endemic of fever by draining the stagnant waters in the neighborhood of the city.

Hippocrates distinguished the intermittent fevers from the continuous, and divided them into quotidian, tertian, and quartan types; he recognized the fact that these fevers are more frequent in the summer and in the autumn and in the vicinity of stagnant waters, especially after a rainy spring season; that they may become malignant, whence the necessity for precaution in the prognosis and treatment; and that in some cases they are followed by affections of the spleen and by dropsy.

In the "Treatise on Medicine," by Aulus Cornelius Celsius, the first medical work of any importance which was written in the Latin tongue, the various types of intermittent fever are described more at

length—that is to say, the quotidian, the tertian, and the simple and double quartan; he distinguishes the simple tertian from another in which the attack is of longer duration and much more severe in character.

In regard to the etiology of these fevers, it is well to know the opinions of several of the ancient writers, some of which seem to be veritable intuitions. Thus Marcus Terentius Varro, in his "Rerum rusticarum," Lib. I., writing of the necessary conditions to be looked for in a country site where one may wish to build a villa, says: "Animadvertendum etiam si qua erunt loca palustria, et propter easdem causas, et quod crescunt animalia quædam minuta, quæ non possunt oculi consequi, et per aera intus in corpus, per os ac nares perveniunt atque difficiles efficiunt morbos."

Columella ("de Re rustica," Lib. I., Cap. 5) writes: "Nec paludem vicinam esse oportet ædificiis nec junctam militarem viam; quod illa caloribus noxium virum eructat et infestis aculeis armata gignit animalia, quæ in nos densissimis examinibus involant."

And Palladius ("de Re rustica," Lib. I., Cap. 7) thus expresses himself: "Palus omni modo vitanda est, præcipue quæ ab austro vel occidente, et siccari consuevit æstate, propter pestilentiam, vel animalia inimica, quæ generat."

From these quotations, to which we might add many more, it is evident that these writers were convinced that marsh lands were injurious to the health of man by reason of the miasms, invisible animal life, and insects emanating from them. Through the centuries which followed, these ideas were lost from time to time, but reappeared and remained in the ascendant up to the time of the discovery of the parasite.

The clinical study of malarial fevers received a progressive impetus in the second half of the seventeenth century from the work of Ludovico Mercato ("Opera Medica," Venetiis, 1608), physician to Philip II. and Philip III., kings of Spain. He was the first to describe the pernicious intermittent fevers of tertian type, and he divided them into six forms, basing his classification less upon the syndrome than upon abstruse theoretical considerations.

Other writers following him speak of pernicious fevers; among these Riverio ("Opera Omnia," Lipsia, 1640), professor in the University of Montpellier, who in describing pestilential fevers, includes among them the malignant tertian which are usually fatal at the third or fourth attack.

The introduction of cinchona bark into Europe towards the middle of the eighteenth century marks the end of the ancient period in the history of the researches into malarial infection.

The value of cinchona bark in the treatment of distinctly intermittent malarial fevers was very quickly recognized; and it soon became an important factor in the distinction of these so-called essential fevers—that is to say, in the separation of those which are curable by cinchona bark from those which are unaffected by it. After the discovery of the febrifuge properties of cinchona, four renowned physicians devoted their attention to this class of fevers, namely, Sydenham, Morton, Torti, and Lancisi.

Sydenham of London ("Opera Medica," Genevæ, 1723), whose works upon intermittent fever are well worthy of study, gave an admirable account of these fevers, endeavored to ascertain the cause of the attacks, and described the treatment by cinchona bark. Later he called attention to the pernicious fevers which occur and which give apoplectic symptoms, and said that they should not be treated by the remedies then in vogue, but by cinchona bark.

Morton, also of London ("Opera Omnia," Lugduni, 1697), entered more minutely into the description of pernicious fevers and the many varieties of symptoms which characterize them; he recognized the fact that they, as well as the simple intermittent fevers, should be treated by cinchona bark, which he considered as an antidote to the fever poison. To Morton also belongs the credit of recognizing the fact that these fevers are produced by emanations from marshy regions, especially in the autumn.

Torti wrote a book ("Therapeuticæ specialis ad febres periodicas perniciosas," Venetiis, 1753) which, although bearing the modest title of the "Treatment of Pernicious Fevers," is really a complete clinical treatise upon intermittent and subcontinuous, simple and pernicious malarial fevers. Of the various forms of pernicious infection there is given a clinical description so clear and so accurate as to demonstrate the profound and judicious spirit of observation and the broad experience of the writer. Torti's book may be read with pleasure and profit at the present day. Mannaberg in his recent treatise upon malarial infection quotes largely from this book, just as we ourselves have done in previous works and shall do in this.

While the authors so far mentioned, with the exception of Morton, concerned themselves little or not at all with the external origin of intermittent fevers, Lancisi, the physician to Clement XI., in his work entitled "De noxiis paludum effluviis" (Rome, 1717), turned his attention to this neglected subject, inspired perhaps by the ancient Roman writers whom we have mentioned. Lancisi tersely states that the cause of simple and grave intermittent fevers is to be found in the regions where there are natural or artificial marshes, as demonstrated by the disappearance of these fevers after hydraulic improvements.

He mentions the seasons in which the marshes are noxious, and the conditions under which a marsh is injurious to health; he also refers to the immunity possessed by certain inhabitants of marshy regions. Marsh-lands, according to Lancisi, are noxious because of the emanations from them, which he divides into inorganic and living organic. By the latter he means the numerous minute insects which develop in marshes, including the mosquito, of the chief facts in the life of which he makes a special study. Lancisi was the first to attempt to study experimentally the composition of the air of marshes with the view of ascertaining whether there existed in it any minute insect life to be seen only by the microscope. He asks, "An inter animata paludum effluvia sint aliqua cœteris minutioria, quæ intra sanguinea vasa deterius multiplicanda ferantur," and a little later he adds: "Oportet enim febri laborantibus ex palustri aere identidem sanguinem mittere, ac microscopio eiusmodi insecta, si qua sint, ipso in cruore diligenter explorare, quod hucusque nobis concessum minime fuit."

The researches of Mercato, Sydenham, Torti, and Lancisi into malarial fever represent all the work that could be done in those days, with the physiological and pathological knowledge then existent, the means of research at command, with the "humoral" theories then prevalent, and the speculations which dominated thought, but had no foundation in facts.

The clinical and etiological theories upon intermittent fevers acquired in Europe served as a guide to physicians who took part in the military colonial conquests of England, especially Lind and Pringle, in the recognition of these fevers in other parts of the earth, and in the introduction of the study of their geographical distribution.

At the beginning of the present century the various systems of medicine which followed each other swallowed up in the vortex the theory of intermittent fevers. Thus the school of Broussais, denying the theory of humoral intoxication held by the ancient physicians, and basing their views upon inaccurate observations and interpretations of pathological anatomy, held that intermittent fevers were the result of an inflammatory process of the digestive apparatus, to which was added inflammation of the spleen and also of the liver and of the brain in pernicious fevers. The phlogistic theory of malarial fevers caused the physicians who held it wholly or in part to lose faith in the efficacy of cinchona bark and to believe instead in bleeding.

The disastrous results of this theoretical and therapeutic system, as seen especially in Algeria in the first years of the French occupation, have been vividly described by Kelsch and Kiener, who also speak of the good work performed by Maillot, who, repeating in Algeria the studies of Torti, taught the treatment of intermittent and

subcontinuous fevers by the salts of quinine which had been discovered a few years previously by Pelletier and Caventou.

But even the etiology of intermittent fevers, as understood by the ancients and by Morton and Lancisi, was combated; there were writers (Folchi, Oldham, and others) who denied that there was any specific miasm emanating from marshy lands, and held that the fever was due to atmospheric conditions in these regions, especially to the notable difference in the day and night temperature which induced the peculiar perturbation of the vascular and nervous systems, causing the febrile attacks.

In opposition to those who lost themselves in vain discussions upon the etiology of intermittent fevers and the explanation of the varied rhythm of the successive attacks, a few observers added to the doctrines of the ancients theories which recent researches have shown to have been in whole or in part veritable intuitions. Thus Rasori, in a discussion with Agostino Bassi, says: "For many years I have been of the opinion that intermittent fevers are produced by parasites, which re-create an attack in the act of their reproduction, this occurring at more or less rapid intervals according to the species."

And Metaxà writes: "There is no objection to the belief that the parasites of intermittent fever, the first generation of which is exhausted in the first periodical attack, may go on to a second generation in the same body." Farther on he adds: "The duration of the attack is equal to the life of the parasites."

Subsequent studies in pathological anatomy established the anatomical characters of malarial infection (melanosis of the spleen, liver, brain, etc.) through the researches of Meckel, Virchow, Frerichs, and others, and the investigations of other scientists showed the alterations peculiar to other infective fevers, as typhoid. Thus pathological anatomy allied itself to clinical experience and to epidemiology in the work of differentiating between malarial infections and other febrile infections which were easily confounded with the subcontinuous form of the former.

In spite of all this, confusion reigned. Some forms of typhoid fever were held to be malarial; some authorities held that dysentery and yellow fever were results of malaria; in malarial regions complications of endemic infections with other diseases were seen where they did not exist, and new diseases were created, such as typho-malarial fever. A consequence of all this was the terrible abuse of quinine which was used to overcome the supposed malarial infection or complications to such an extent as to shake one's faith in the specific value of the remedy.

In the latest works upon malarial infection prior to the discovery

of the parasite, among which were those of Baccelli, Fayrer, Hertz, Kelsch and Kiener, and Sternberg, the attempt was made, chiefly by clinical observations, definitely to circumscribe the limits of the fever of malaria in order to distinguish it from other infections.

On the other hand, the conviction that a malarial parasite certainly existed had induced several investigators in a short space of time and in various places to institute researches in that direction. After many vain efforts upon the part of others, the malarial parasite was discovered by Laveran towards the end of 1880. But his discovery was not universally acknowledged for several years; not, indeed, until after the researches made in Italy had enriched the parasitology of malarial infection in man with new data, thus completely developing this branch of science.

The parasite thus discovered did not belong to the class of bacteria among which had been found many of the pathogenic agents of several infective diseases, but to the protozoa. This was the first example of a protozoic infection in animals, although phytopathology had shown that there were plant diseases caused by analogous endocellular parasites. Later endoglobular parasites like those of human malaria were found in many animals.

While the researches into malarial parasitology were going on towards perfection, clinical and anatomico-pathological studies were taken up with ardor, and the result was the solving of many scientific and practical problems which had been the subject of investigation for centuries. If to these results we add the most recent ones upon the life of the parasite outside of the human body and upon the way in which man takes the infection (matters of supreme importance in prophylaxis), we have grounds for the assertion that a greater and better scientific edifice could not have been erected in so short a space of time.

PARASITOLOGY.

The malarial parasites of man belong to a numerous family of beings that live and are developed within the red blood cells of many kinds of animals. They are known to exist in the red corpuscles of reptiles, amphibia, birds, and mammals. For all these parasites the name of hæmosporidia has been adopted by many.

The natural history of these beings is complicated. If we limit ourselves to the consideration of the best-studied species, namely, the parasites of warm-blooded animals, we may hold it to be a well-authenticated fact that they possess two life cycles, one being completed within the red cells of the warm-blooded animal and the other in the tissues of an insect.

Thus the parasites of Texas fever (bovine malaria) live in the red cells of cattle; from these they pass into a special kind of tick (*Boophilus bovis*, of Riley), then from the infected mother tick to its progeny, and this by pricking a healthy ox communicates to it the infection. So also with the parasite known as "proteosoma" which lives in the blood of birds; from these it passes into the middle intestines of a special kind of mosquito (*Culex pipiens*), where it goes through a whole life cycle, ending in the salivary glands of the mosquito, and when the latter stings healthy birds, their blood is in time infected.

In the same way the malarial parasites of human beings develop and multiply in the red blood corpuscles of man, where they go through an undetermined number of life cycles, and thence pass into the middle intestine of certain species of mosquito (*Anopheles claviger*, for instance), in which they go through the various phases of a new life cycle which ends in the poison-salivary glands; from these, when the mosquito bites in order to obtain nourishment, the parasite passes again into man.

We have therefore two cycles of life to study in these parasites—one which is completed in man and the other in some species of mosquito. We will here give an outline of these biological cycles which we shall study in detail in the following chapters.

The phase of life which is completed in man is the cause of the malarial fever. In this phase the parasites, in their young stage, appear as very small amœboid bodies endowed with more or less rapid movement and which exist within the red blood corpuscles, by the substance of which they are nourished, converting the hæmoglobin into black pigment; as they are nourished they increase in size and lose a little of their motility and (still within the globules) multiply by a process of fission. The daughter cells resulting from this fission become free in the plasma and invade other red corpuscles in which they begin the same life cycle.

To this life cycle are intimately related the two salient morbid phenomena of acute malarial infection, intermittent fever and anæmia. The first is manifested when the parasite is undergoing multiplication; the second is produced principally through the destruction of a large number of red blood cells, which have gone in part to nourish the parasite. In all malarial parasites this cycle is completed in essentially the same manner. The structure is also fundamentally the same in all; it consists of a vesicular nucleus furnished with one or more small specks of chromatin, and by a ring of protoplasm which during its development becomes pigmented by black granulations (melanin), representing the residua of the digestion of hæmoglobin. The process of multiplication is identical in all: the chromatin in-

creases in volume and is divided into a number of tiny bodies; thus is formed a varying number of new nuclei, around which the segmenting protoplasm disposes itself. When the formation of the daughter bodies is finished, there remains a "residuum of segmentation" composed principally of black pigment.

But while the life of these little beings is developing there are some differences to be noted in the various malarial parasites, which increase with the progress of development. These differences, especially in the young forms, relate to the motility, which may be of several degrees; the minute characters of the pigment with which the protoplasm is loaded; the retrogressive changes undergone by the invaded corpuscles; the length of time necessary for the complete development of the parasite; the number of daughter bodies resulting from the division of an adult parasite, and a few details in regard to the manner in which fission occurs.

These differences constitute the morphological basis for the division of the parasites into various distinct species, which have constant characteristics and do not become transformed from one into another. It has from the earliest days been known that malarial fevers present different clinical characters, which permit of their being divided into various clinical groups or species. Recent researches have demonstrated that each species of malarial parasite is the cause of a special kind of malarial fever, so that simply by examining the blood of a patient we can authoritatively state the kind of malaria from which he is suffering. But in addition to the life cycle whose general outlines we have given and which is entirely completed in man, every kind of malarial parasite has another which only begins in man. Some parasitic bodies increase in size without dividing until they form bodies of characteristic shape and structure larger than a red blood corpuscle. These bodies circulate in the blood for several days, without giving rise, when they are alone, to any morbid phenomena, such as fever or anæmia; then, remaining sterile, they degenerate and disappear. If the blood is subjected for a certain time to examination under the microscope, we shall find that some of these bodies throw out flagella which move with great rapidity, and becoming liberated move around the red corpuscles with vivacity, whereas others do not present this phenomenon. This phase of life is represented by special bodies characteristic of one species of malarial parasite (the estivoautumnal) called, from their appearance, crescent bodies.

The significance of these bodies has been the subject of much discussion among various investigators, and it is only of late, and after a long series of erroneous conclusions, in some of which, however, there was a glimmering of the truth, that we have now definitely

ascertained that these bodies, which when they remain in man degenerate and disappear, are capable of further development when they pass into the intestines of certain species of mosquito. From them starts the second life cycle, to which we have alluded above, a cycle which, thanks to very recent researches, we can describe in outline as follows: When a mosquito of the right kind bites a sick person in whose blood are the crescent bodies or their homologues in other species of malarial parasites, some of these are taken in with the blood; then in the mid-intestine of the mosquito certain crescent forms give out the so-called flagella which are motile filaments provided with chromatin; these filaments fecundate other crescent forms, which at this point become capable of penetrating the epithelium of the mid-intestine and of travelling between its muscle fibres.

According to all recent researches, there would therefore seem to be a differentiation of sex in the crescent bodies and their homologues in the other species of malarial parasites. Those becoming flagellated represent (to follow the nomenclature of the zoologists who have described similar sexual phenomena in other sporozoa) the *microgametocytes* (cells producing the male elements), while other non-flagellated bodies are the *macrogametes* (female element). The fecundated macrogametes undergo their further development between the muscle fibres of the small intestines of the mosquito; then they take on a capsule, assume the aspect and characteristics of typical sporozoa, and increase progressively in size until they project into the cavity of the celœma; at the same time the nucleus divides into a great number of nuclei which become smaller and smaller, each one of which becomes the nucleus of a *sporozoite*. This latter is a small filament with very slender and usually curved extremities, having at its centre a little nucleus long in shape and furnished with a few rods and granules of chromatin.

At this point the capsules of the sporozoa break and the sporozoites become scattered throughout the body cavity. Many of them collect within the cells in some of the tubules of the salivary glands of the mosquito, and when the insect again stings a human being, they are inoculated together with the irritating secretion of the gland. This cycle, from the small intestine to the salivary gland, is accomplished in a varying length of time, from eight to ten days or more, according to the temperature of the surrounding atmosphere. It would seem, moreover, that there are some slight differences according to the species of malarial parasite.

Up to this point, guided by the facts observed, we have been able to construct the complicated biology of the malarial parasite. The parasites pass from man to the malarial mosquitos and from these

to man again with alternating generations. The cycle completed within the mosquito being characterized by forms of a higher development (encapsulated forms), the malarial mosquito must be considered a definitive lodging-place for the parasites; that in man being characterized by a lower grade of development (amœboid forms) must be held to be an intermediary abode.

It is, however, more than likely that these theories do not as yet reveal to us the whole of the life of the malarial parasite. Is this double cycle enough to insure the indefinite preservation of the parasites? In other words, is the presence of man absolutely indispensable to this preservation? Can the malarial parasites of man be found in other warm-blooded animals, so that even were man absent they would be able to complete the double life cycle? Again, can the malarial parasites pass from the infected mosquito mother to the egg, and thence to the larva and the new generation of winged insects, as the parasites of Texas fever pass from the infected tick to the young generation of ticks? Researches are still being pursued along these lines and based upon theories suggested by the difficulties in explaining certain epidemiological data by the known facts; it is therefore quite useless for us to prolong a discussion upon the comparative value of the various theories, when it may be that in a short time we can speak with the authority derived from known facts.* The following pages are concerned with the exposition of the facts upon which are based this synthetical reconstitution and the special study of the various species of malarial parasite, and with the structure and development of each species in man and in the malarial mosquito.

Life Cycle of the Malarial Parasite in Man.

History.

It would be difficult for one who has not followed the development of our present knowledge of the causes of malaria, through the great number of original works published during the past twenty years, to form an exact idea of the way in which, starting from the first incomplete and uncertain researches, we have succeeded in gradually building up a relatively complete and firm structure; nor could he readily distinguish just what part in this laborious work of construction belongs to the various observers.

* The researches carried out since the above was written are held by some to prove that the two life cycles (in man and in the mosquito) are sufficient to explain the known facts, and that hereditary infection in the mosquito does not occur.

Neither would it be easy for one who has not given prolonged attention to these studies to distinguish the known facts from the theories, to recognize the unfilled gaps which yet remain, and to form an opinion among the contradictory views which as to some details are still under discussion.

To a great extent the contradictions originate in the tendency of the various observers to construct a theory and then to take account of their own observations only, without giving due value to the researches and observations of others. But for the present we will leave theories aside and concern ourselves chiefly with well-substantiated facts.

It would not, however, be just to leave unmentioned the researches of Klebs and Tommasi Crudeli, although they possess a merely historical value. It is well known that these writers, in a memorial published in 1879,[1] announced the discovery of a sporiparous bacillus which they called the "bacillus malariæ" in the malarial regions, marshy and non-marshy, of the Roman Campagna. Although all ulterior researches have demonstrated the fact that this microorganism possesses no pathogenic powers, so that it now seems needless to record the various phases in the discussion between supporters and opposers of the bacillus malariæ, yet it is only fair to recall the fact that to these authorities belongs the credit of having begun and promoted the study of malaria by the modern methods of investigation.

We now come to the new period in the history of malaria, that in which the researches were principally devoted to a study of the malarial blood and led to the discovery of the parasite.

It is known that for a long time the attention of investigators had been drawn to malarial blood as that which, among the various changes induced by the malarial agent upon the human organism, presents the most characteristic alteration—melanæmia. Meckel and Virchow[2] were the first to find numerous pigmented cells in the blood and spleen of individuals who died after repeated attacks of malarial fever. Later Frerichs[3] described melanæmia and its effects upon the organism with greater accuracy; in the blood of malarial patients he observed black granules and molecules and pigmented cellular elements which sometimes resembled leucocytes and were sometimes fusiform or cylindrical. He further described accumulations of black granules held together by a pale substance or with an involucrum of a hyaline substance which was sometimes very thin and sometimes rather thick, and pigment masses which were sometimes cylindrical with parallel surfaces, surrounded by a more or less large zone of a translucent substance, occasionally visible on one side only.

Leaving aside other researches which, as they specially concern

the origin of the melanæmia, will be referred to when that subject is considered, our attention is arrested by the observations of Kelsch,[4] who in a detailed work collected the observations made upon many malarial patients in the Philippeville hospital.

Kelsch observed in the blood of many malarial patients, especially those suffering from pernicious fever, the presence of black pigment, either free or enclosed in hyaline masses, or more often in white corpuscles. He noted that the melaniferous cells sometimes held many pigment granules arranged in wreath form at the periphery of the cell; he also described certain other little bodies which possessed a brownish reflection in the marginal zone, and in which were sometimes found fine black granules; and in the blood of the portal and splenic veins he saw melaniferous cells of various shapes and sizes—*i.e.*, spherical, polyhedral, elongated, ovoid, biscuit-shaped, etc.

Thus by the study of the pigmented cells existing in the plasma it was gradually found possible to distinguish from the pigmented leucocytes other bodies of hyaline appearance containing granules of pigment and differing from the leucocytes. But as to the origin, the significance, and the nature of these cells, no one of the authorities referred to had published any opinions worthy of note. To Laveran[5] belongs the credit of having given an accurate description of the pigmented cells described in part by Frerichs and by Kelsch, and of having divined and upheld their parasitic nature. He described three forms of these pigmented bodies.

Bodies No. 1.—These are elongated cells, pointed at the extremity, almost always curved and crescentic, about 8 to 9 μ long, 3 μ wide. Their contour is indicated by a fine line; the body is transparent and colorless, except in the centre, where there is an accumulation of black pigment; on the concave side we often see a fine line which joins the extremities of the crescents.

Bodies No. 2.—These are spherical transparent cells about the diameter of red blood corpuscles, containing pigment granules, which during rest are often arranged in a regular circle, and in movement become violently agitated. Sometimes at the periphery of these cells we see very fine filaments which seem to be inserted in them, and which move most rapidly in every direction, and which sometimes have a slightly swollen extremity. These filaments may detach themselves, and when free move with rapidity among the red cells.

Bodies No. 3.—These are spherical cells, irregular in shape, transparent and finely granular, from 8 to 10 μ in diameter, containing pigment granules, which are sometimes arranged irregularly at the periphery, sometimes gathered together either at the centre or in a

point of the periphery. These bodies are immovable, and according to the author represent the cadaveric form of Bodies No. 2.

Laveran further described in the blood spherical transparent cells containing motile or immotile pigment granules and elements smaller than the preceding, which may be isolated, or united, or adherent to the red cells and to the leucocytes; finally he noted the presence of round, shining motile bodies, without special characteristics, of red cells which seemed to be pierced in one or more places, and which contained pigment granulations, and of free pigment granules derived from the destruction of parasitic elements.

Laveran held that all these bodies represented several stages in the existence of one parasite, which appears in an encysted state, and in its perfected form becomes free as a motile filament.

Bodies No. 2 are the cysts which contain the filaments.

Richard[7] confirmed Laveran's observations in full, but instead of holding with him that the small pigmented forms are found adhering to the red corpuscles, he thought that they were contained within them, developed there, grew, and finally, when perfect, left them. He described these pigmented bodies in the various stages of development within the red corpuscles up to the time when, having reached a state of maturity, they perforate the membrane of the red cell, become free in the blood, and are then sometimes furnished with very mobile filaments, like the bodies described by Laveran. Later he abandoned this view and indorsed that of Laveran.

After the publication of the results of the first researches by Marchiafava and Celli[8] regarding the alterations of the red cells in malaria, which we shall describe later, Laveran, in his "Traité des fièvres palustres,"[6] repeated and amplified his first descriptions of malarial parasites. In regard to the cystic Bodies No. 1, he added that sometimes they present a double outline—this same double outline he occasionally found in Bodies No. 2. As to the mobile filaments, he said that it was possible that there was a canal in them through which a small particle of plasma or of pigment might be introduced, which, becoming arrested at some special point in the filament, usually at its free extremity, produced the swelling so often seen. He persisted in believing that these filaments were contained in Body No. 2 as in a cyst, and that Body No. 3 was a cadaveric form of Body No. 2; in them he sometimes saw the pigment in the centre and around this a distinct sign of segmentation of the plasma.

He referred, moreover, to movements of cyst Body No. 2 or the spherical bodies, which he compared to amœboid movements (page 165), describing them as follows: "When we examine medium-sized or large Bodies No. 2 at a temperature of from 30° to 35° C. (86° to

95° F.) we often find that these bodies become changed in shape, while the pigmented granules within them are moving with great rapidity. These deformations, which are produced somewhat slowly, like those of amœbæ, are very easily perceived if we are careful to keep the same cells in the field of vision of the microscope, and to make a sketch of them every five minutes" (page 168). Finally he called attention to the clear spots in some of the red corpuscles: "In the preparations of malarial blood we often find red corpuscles which have small, clear spots and which might be called *hématies piquées*. It is probable that these clear spots are produced by spherical bodies in a nascent state, so to speak, which do not yet contain pigment. Marchiafava and Celli appear to have observed this phase in the alteration of the blood of malarial patients."

We have quoted these sentences in full, because in Laveran's later works they have been several times cited in order to prove that all that has since been found and said has been simply confirmatory of his discovery. We will not here go into the controversy which has arisen upon this question. Marchiafava and Celli have discussed it in an article published in 1888 in the *Archivio Italiano di Biologia;* and to this article we refer any reader who may wish to pursue the subject further. To the unprejudiced reader it will certainly seem to be proved by all the facts of the case that Marchiafava and Celli were the first to observe and describe the young, non-pigmented malarial parasite within the red corpuscles, to recognize that it was endowed with very rapid amœboid movements, to demonstrate its staining properties with aniline colors, and to call attention to its importance in those forms of malarial infection in which it is the chief element present, not only in the peripheral blood, but also in the cerebral capillaries in some cases of pernicious fever.

This first series of researches into the nature of the pigmented bodies found in malarial blood began, therefore, with the recognition of morphological differences between these bodies and the pigmented leucocytes, and ended with the belief in an extraglobular pigmented parasite whose perfected form was the mobile filament of Laveran.

At the same time other investigators had in other directions begun to inquire into the alterations of the red cells in malaria. Already Kelsch in a numerous series of observations had demonstrated that few diseases produce so rapid and so grave an anæmia as malarial infection; twenty days of fever may suffice to reduce the number of red cells of a patient from five million per cubic millimetre to one million or even less. Later Marchiafava initiated the study of the malarial changes in the red blood cells by examining the bone marrow and the splenic pulp of melanæmic infants; and he came to the

conclusion that the red cells do not give rise to the formation of black pigment after their disintegration, but that, on the contrary, the conversion of the hæmoglobin into black pigment occurs gradually within the corpuscle itself.

The later researches of Marchiafava and Celli [9] conclusively demonstrate the truth of this view; from the date of their first article upon the subject they firmly established these two facts: 1. In malarial infections the red corpuscles contain to a greater or less extent (in pernicious fevers sometimes to an enormous amount) little bodies which in dried preparations are deeply stained by methylene blue, and which in form and size possess a certain resemblance to micrococci. 2. The formation of pigment in melanæmia occurs in the circulating blood within the red corpuscles by the transformation of hæmoglobin into melanin after the appearance of the above-mentioned little bodies.

Whatever the nature of the alterations described, the writers demonstrated their great importance in the diagnosis of doubtful cases of the disease.

In the same year (1883) these writers gave a more detailed description of these alterations in a work upon the origin of melanæmia[10] in which they propounded the inquiry as to whether these little bodies which are stained by methylene blue should be considered as parasites or as the product of a slow necrobiosis of the red cells; they endeavored to answer the question by raising cultures in various nourishing media and under varying conditions, but without results, and they held to their last theory. In this work they gave a minute and accurate description of the varying aspect of these little bodies in the stained preparations and gave a sketch of the annular forms (Fig. E), which, as was recognized later, are the typical forms of young malarial parasites that are properly fixed and stained.

The idea that these small bodies described in 1883 were parasites became more than a mere probability to these authors when they systematically studied fresh preparations of the blood without the use of coloring matters or of any reagent. In a work published in 1885 [12] they showed that the bodies, when stained and under a one-twelfth Zeiss objective, could be clearly distinguished from the accidental vacuoles in the red corpuscles. They appear, in fact, as small endoglobular protoplasmic masses of varying size, with or without a central vacuole, and endowed with lively amœboid movements which cause them continually to change their form. They also described the amœboid movements of the pigmented endoglobular bodies, and followed the endoglobular development of the crescent bodies. They observed the flagellated bodies of Laveran, the filaments of which

they held to be flagella, and not parasites which had emerged from a cyst; in addition they perceived various bodies whose outlines moved with rapid undulations, and various methods of division of the pigmented bodies. In regard to the last, they expressed the opinion that it represented a method of multiplication of the parasites. From all these facts they formulated the opinion that the small amœboid bodies were parasitic protista which developed within the red corpuscle and transformed the hæmoglobin into melanin.

In the same work the authors reported five cases of experimental malarial fever obtained by the injection of malarial blood; in four of these the characteristic amœboid parasites within the red corpuscles were found from the very onset of the fever, thus proving the malarial nature of the infection caused.

In another publication[12] the preceding researches were in large part confirmed and amplified. The authors described a division of non-pigmented endoglobular parasites, repeating their opinion that these as well as the form of division of the pigmented bodies represented a mode of multiplication of the parasites, and for these parasites they proposed the name of *plasmodia* or *malarial hæmoplasmodia*.

At this point the French and Italian observers met upon the same ground, in spite of their different starting-points and their differing points of view. The pigmented parasite of Laveran is merely a stage in the development of the endoglobular plasmodium. Thus has been established the doctrine of endoglobular parasitism in malarial infection and with it the doctrine of melanæmia.

Those who have recently taken up the history of our ascertained knowledge of the malarial parasite have not brought into sufficient relief the demonstrative value of these first researches of Marchiafava and Celli. In point of fact they furnished the first scientific data which enabled us to believe, with any true basis for the belief, in the parasitic nature of the bodies described in the blood. They demonstrated (1) that these bodies are constantly found in one or another form in all cases that can be diagnosed with certainty as malarial; (2) that they are found in no other infection; (3) that they can be clearly distinguished from the retrogressive alterations of the red blood corpuscles; (4) that they multiply by means of fission.

In support of their views these authors adduced the analogous phenomena of endocellular parasitism described in certain plants (as by Woronin and Zopf, for instance), at a time when there were no examples of similar facts (endocellular parasitism) known in human pathology or that of the higher animals.

The value of these demonstrations, which are quite wanting in the researches of Laveran and Richard, in whose writings we find a con-

cise and correct affirmation without the slightest attempt at scientific demonstration, was not unnoticed by contemporary scientists who had followed the development of the discovery step by step. A proof of our view of the matter is found in the fact that those who believed in the existence of a bacillus malariæ, as well as the upholders of the purely degenerative nature of the changes in malarial blood, all, or nearly all, directed their arguments against the researches of Marchiafava and Celli. We cannot discuss with those who merely make an affirmation, but with those who attempt to prove their statements.

In fact, while on the one hand the effort was being made to obtain the above-mentioned results, on the other hand various observers expressed a doubt as to whether the endoglobular bodies described as the parasites of malaria did not merely represent a degenerative change of the red corpuscles. Hæmatologists such as Ehrlich [28] suspected that the Italian investigators had described as parasites the irregular spots which in many red corpuscles can be stained by methylene blue; physiologists such as Mosso[29] claimed to have reproduced forms not to be distinguished from the malarial parasites by injecting the blood of a dog into the peritoneal cavity of a hen; clinicians such as Maragliano held that the parasites were not to be distinguished from the alterations that are produced in all blood kept under abnormal conditions; zoologists like Grassi [30] saw in the new endoglobular parasites only a "falling star."

Then followed a long series of discussions which were carried on for several years and of control experiments which were not without value, because while on the one hand they served to strengthen the conviction of the accuracy of the observations made upon malarial blood, on the other they promoted the study of the retrogressive histological changes in the red blood corpuscles.

The last lingering doubts, if any such existed, were soon dispelled by the rapid progress in the knowledge acquired as to the biology of parasites in relation to the various kinds of fever and by researches into the intimate structure of the parasites themselves.

The first series of researches soon bore fruit of the greatest value. The various investigators of the malarial parasite working in different seasons and countries—that is to say, in countries where malaria is mild and in those where the type is grave—soon perceived that the results obtained were not entirely harmonious. Some found a predominance of a certain form of malarial parasite, as, for instance, the small plasmodium with little or no pigment, others the variety which speedily develops into the large pigmented body. This promptly suggested the thought that various species of parasites corresponded to various types of the fever. Golgi was the first to turn his re-

searches in this direction, and taking up the quartan first of all, he gave an exhaustive description of this form of fever. [14] He next systematically studied the tertian,[15] calling attention to the difference in the parasites of the two kinds of fever. At the same time he established the relationship between the cyclical development of the parasites and the clinical course of the fever, showing that the onset of every febrile attack corresponds to the multiplication of a generation of parasites.

With the same object Marchiafava and Celli studied the type of fever which predominates in the Roman Campagna in the summer and autumn (grave fevers), which they found to be in relation with the cycle of development of the small amœboid parasites.[13] Researches in the same direction were afterwards instituted by Golgi,[16] Canalis,[18] Antolisei and Angelini,[19] Grassi and Feletti,[20] Bignami and Bastianelli,[21] Vincenzi, and others.

In 1891 Marchiafava and Bignami[23] isolated from the group of estivoautumnal fevers the estivoautumnal tertian or malignant fever, describing its clinical type and special parasitic characteristics.

From these researches, confirmed later by the majority of accurate investigators, as Mannaberg, Thayer and Hewetson, and others, has arisen the doctrine of a plurality of parasitic species, a view upheld by nearly all Italian authorities against the unitary theory of Laveran. This view, which may well be called the Italian view, originating from clinical and parasitological considerations, received support from the results of experimental inoculations upon man, performed for the most part in the medical clinic of Rome by Baccelli and his assistants, among whom Antolisei should be given the chief place. These inoculations demonstrated that the various forms of parasites to be distinguished morphologically are reproduced in the person inoculated in the same form and with the same type of fever, and are not capable of being transformed the one into the other; this demonstration was given for quartan, tertian, and estival fevers, and recently by Bignami for the estival tertian.

The researches into the intimate structure of the parasites which were carried on by many investigators using many methods, while they gave harmonious results as to the facts observed, led to the most discordant interpretations. We recall in this connection the researches of Celli and Guarnieri, who, concerning themselves chiefly with the tertian parasites, gave an accurate description of the facts, although their interpretation of these facts could not be indorsed. After them came Grassi and Feletti, and Mannaberg, who demonstrated the nucleus of the parasite. Later the method of staining suggested by Romanowsky has enabled him, Ziemann, Bastianelli,

Bignami, and others, to follow better than they could by any other method the various modifications of the nucleus during the process of multiplication. Finally Bastianelli and Bignami, by the use of the same method, have recently succeeded in showing the structure of the flagellated bodies and of the mobile filaments as to which the greatest uncertainty existed.

We have seen that the researches of Golgi explained the problem of the intermittence in malarial fever, a problem which up to that time had been unsolved. Marchiafava and Celli occupied themselves principally with the pathology of pernicious fever, and then Marchiafava and Bignami took up this subject and published the results of their work in 1892.[24] In 1890 Bignami,[25] when studying the anatomical lesions of pernicious fevers, endeavored to explain the latter by the parasitic invasion, and starting from parasitology tried to work up to the interpretation of the pathogenesis of the chief symptoms in grave infections. Guarnieri[27] had already made the same endeavor in regard to the liver lesions. Later Bignami,[26] studying the anatomical lesions of chronic malaria, attempted to ascertain its pathogenesis, starting from the lesions of acute infections. These anatomico-pathological researches were confirmed in all essential points by the later work of Monti, Barker, and others.

In the same way the study of other points relating to the pathology of the infection was promoted, such as the question of relapses, of incubation, of the complications, etc.

The practice of medicine has derived incalculable benefit from all these researches. The certainty of diagnosis in doubtful cases, the positiveness of the opinion which can be given in the presence of complications, represent conquests of science so valuable that only the physician accustomed to the daily uncertainty and experimentation of practice can properly appreciate it. As to therapy, most important data have been acquired in regard to the mode of action of the salts of quinine (Golgi,[17] Marchiafava and Bignami,[24] and others), and we have learned the scientific reasons for certain practices the use of which clinical experience had long suggested to physicians.

Methods of Examination.

The methods of research, of course, vary according to whether the examination is made solely for purposes of diagnosis or to show the special structure of the parasite.

For the *examination made for diagnostic purposes* it is only necessary to examine fresh specimens of blood, but this requires long practice on the part of the physician. He naturally cannot have confi-

dence in his own examination unless he has a complete understanding of the artificial changes which may be found in the red corpuscles of normal blood in ordinary preparations. Only long practice in the examination of blood under both normal and pathological conditions can give the necessary sureness of judgment. This statement will not appear to be an exaggeration if we consider that for many years even physicians expert in microscopic examinations upheld the opinion that the malarial parasite could not be clearly distinguished from the ordinary changes in the red blood cells.

For fresh examinations we proceed as follows: A puncture having been made, after careful cleansing of the skin, in the ball of the thumb or in the lobe of the ear of the patient, a drop of blood is squeezed out by gentle pressure. It is well to wipe off the first drop and use the second in the preparation, placing the cover-glass in contact with the top of the drop and avoiding all contact with the skin. The cover-glass is then turned over upon the slide and gently pressed down so as to spread out the blood in a thin layer. Gentle pressure does not affect the red corpuscles or the parasites enough to interfere with the examination. The preparation may be considered successful if the red blood cells are not arranged in rouleaux, but are spread out singly, so that the observer can examine the contents of each cell. To attain this end the slide and cover-glass must be thoroughly cleansed with alcohol, and the cover-glass must be of the thinnest possible. Then if a very small drop of blood be taken it will spread of itself without pressure. This gives us the best preparations.

An ordinary immersion lens (one-twelfth homogeneous immersion) is used. The pigmented form of parasite is easily seen with a good dry lens magnifying four hundred to five hundred diameters; Laveran's researches were made without immersion. In this case, the finding of the parasite is aided by the presence of grains of black pigment which serve as landmarks, and by the relatively large size of the parasitic forms containing them. But to see and clearly recognize the non-pigmented young parasites in the red corpuscles, to distinguish them from the vacuoles or decolorized spots that may accidentally be found there, a good immersion lens is absolutely necessary. Without it, indeed, it may be impossible to diagnose malaria, especially in primary and grave infections, in fevers which are not distinctly intermittent and in which, for a while at least, we may find only the small non-pigmented plasmodia in the blood, sometimes also in small number. But the certainty of diagnosis may be lacking in just those cases in which, because of the atypical course of the fever and the absence of a history of malarial febrile attacks, the physician feels the most need of recourse to an examination of the blood.

Fresh preparations may be kept for several hours, because very often the stratum of blood which is at the periphery of the cover-glass by its rapid drying prevents further evaporation and consequent alteration of the corpuscles in the centre. They are, of course, preserved even longer in a moist chamber or when sealed with paraffin.

If the examination for diagnostic purposes cannot be made at once, we must resort to drying and staining. For this purpose we take up a small drop of blood upon a very thin and very clean cover-glass and make it spread rapidly by sliding the cover-glass upon another one; if would be well to hold the glasses with specially constructed forceps. We proceed, in fact, in the same way as in making preparations after Ehrlich's method. The blood is let dry in the air, then the cover-glasses are immersed for from twenty to thirty minutes in a mixture of equal parts of absolute alcohol and ether, which is the fixative, after which the staining is done by means of an aniline color or by hæmatoxylin.

The color usually selected for rapid staining is methylene blue in a saturated aqueous solution, or the boracic methylene blue of Sahli, or the methylene blue of Löffler (30 c.c. of a concentrated alcoholic solution of methylene blue in 100 c.c. of a solution of potassa 1:10,000); the second staining may be effected by eosin. Excellent results can be obtained by using a saturated alcoholic solution of methylene blue, as Marchiafava and Celli did in their first researches; in this case it will not be necessary to fix the preparations in alcohol and ether. Even with Ehrlich's hæmatoxylin of a strong staining-power we may obtain a rapid coloration of the parasites, excellent in an examination for diagnostic purposes.

Some claim that puncture of the spleen is in many cases necessary for a diagnosis of malaria; we have never had to resort to it to find the parasite if an examination of the blood of the periphery had been made often enough and with sufficient care. We must, however, resort to splenic puncture for the study of some of the stages in the life of the estivoautumnal parasite (adult forms, fission forms) which are to be seen in the peripheral blood only in the gravest cases. This puncture is to be performed under all aseptic precautions with a well-sterilized Pravaz or Tursini syringe with a long and rather large needle. The patient must hold his breath as the needle is inserted, and by a slight pressure under the left costal arch we push the spleen against the thoracic walls. Although, as we know, there are certain diseases in which hemorrhages are so easily produced that puncture of the spleen might be dangerous, we have never had any accident of the kind in the many punctures which we have made in cases of malaria, not even in the grave cases in which the spleen was much

softened. The puncture, however, must always be performed with great precaution, especially in pernicious fevers. If during the puncture the patient takes a rapid breath, the needle will perforce slightly lacerate the capsule, an occurrence which is to be avoided if possible.

For the *study of the structure of the parasite* various methods have been adopted. Some have tried to stain the parasite in ordinary fresh preparations (as Celli and Guarnieri, Grassi and Feletti); others stain them in the preparations previously dried by the Ehrlich method. We shall refer to a few of these methods only.

Celli and Guarnieri proposed to stain the malarial blood by adding to a drop of it a drop of a saturated solution of methylene blue dissolved in ascitic fluid. By this method they were able to see very clearly the structure of several phases in the tertian parasite especially; but with the forms of parasites undergoing multiplication, the crescent forms, etc., the image is not clear enough to show the structure.

Grassi and Feletti have suggested several methods. In their opinion the best results are obtained by first staining in hæmatoxylin the preparations which have been dried and fixed in absolute alcohol and ether, to which have been added a few drops of acetic acid; second, the coloring of fresh preparations with methylene blue, which is done by mixing a drop of blood with a drop of a dilute aqueous solution of methylene blue. By this last method we clearly perceive the nucleus with its delicate membrane and the so-called *nucleoliform node* which takes on a deep color. But the parasites appear to be swollen and dropsical, and the red corpuscles are altered by the action of the distilled water, so that, although the preparations are demonstrative so far as the nucleus is concerned, they do not show with any distinctness the minute structural details.

Mannaberg has proposed staining with hæmatoxylin after fixation in picric acid. He uses preparations of blood dried in the usual way and washed in a dilute solution of acetic acid to remove completely the hæmoglobin from the red corpuscles, after which the preparation is fixed in picric acid (concentrated solution of picric acid 30, distilled water 30, acetic acid 1) for two hours, and then passed through absolute alcohol. The staining is done by alum hæmatoxylin and takes from twelve to twenty-four hours; finally it is differentiated by hydrochloric alcohol 0.25 per cent. and ammoniated alcohol (3 drops of ammonia to 10 c.c. of alcohol), washed in alcohol and mounted in xylol balsam.

Our preference has usually been for the fixation of the preparation in equal parts of absolute alcohol and ether, and staining it with Ehrlich's hæmatoxylin and with eosin. As regards the parasites,

the results obtained are the same as by Mannaberg's method, but the red blood cells are more perfectly preserved. The nucleated cells and the phagocytes with their changes, etc., are well seen. As to the structure, although it is distinctly visible, by none of these methods are we able to follow its modifications, the arrangement of the chromatin, etc., in the bodies just preparing to multiply, nor have these methods given good results in the attempt to study the crescents and the forms derived from them. The reason for most of the contradictory results obtained by various observers in the study of the internal structure of the malarial parasites, and especially of the manner in which they multiply, is to be found in the defectiveness of the methods used, which do not permit of the nuclear chromatin being clearly distinguished from the cytoplasm (at least in certain phases of the life cycle of the parasite), the latter being deeply stained by hæmatoxylin and nuclear stains in general.

For a more minute histological examination it is absolutely necessary to have a method which will give a specific color to the chromatin entirely different from that of the protoplasm. Romanowsky's method meets this indication, and is so much superior to all others that we will give a brief description of it.

The coloring substance used by Romanowsky consists in a mixture of methylene blue and eosin in definite proportions. When to a saturated solution of methylene blue we add a one-per-cent. solution of eosin, there is a moment during which the commencing formation of an insoluble precipitate imparts a violet tint to the fluid. The mixture in which this precipitate begins to form consists of one part of the concentrated methylene blue solution and two parts of the aqueous one-per-cent. eosin solution, but this proportion may vary according to the quality of the stains employed. For this reason it will be necessary to experiment with them until the exact proportions of the mixture have been determined. When the mixture is quite perfect, Romanowsky claims that a special neutral stain will be formed which has marked affinity for the chromatin of the nucleus; and the moment of formation of this third color is that of the formation of the precipitate, at which time the mixture possesses its maximum elective staining-power. Like Ehrlich's mixture, it is to be used without filtration, the watch-glass being kept well covered to prevent evaporation. Upon the surface of the liquid (after having carefully removed a violet scum with metallic lustre which forms on it, so as to avoid the formation of a precipitate upon the glass) we float a cover-glass, on which the blood is dried after Ehrlich's method after having been previously fixed in alcohol for half an hour. In from one-half to one hour the staining is accomplished, but if a very

strong coloration is desired, it will be better to let the action continue for two or three hours. The preparation is then washed many times in distilled water, dried, and mounted in the usual way. The stain must be prepared each time, and can be used once only.

By this method the red blood cells are stained pink by the eosin, the nuclei of the white cells a dark violet, the protoplasm of the eosinophile leucocytes a deep pink, that of the neutrophiles a pale violet with purple granulations, that of the lymphocytes and large mononucleated cells blue; the plaques are a violet red; the protoplasm of the malarial parasites is of a more or less intense blue, and the nuclear chromatin a violet red, which permits us to follow the modifications of the nucleus during the development of the parasites. The best results are obtained by this method when the stains are of good quality and mixed in the right proportion. Ziemann, who has applied it not only to the study of malarial parasites but also to that of other microorganisms, prefers the Methylenblau medic. puriss. (Höchst), the Methylenblau rectificat. of Grübler, and eosin A. G. and B. A. (Höchst). In preparing the mixture he prefers to use a one-per-cent. solution of methylene blue and a 0.1 per cent. of eosin; the desired proportion between the two solutions is usually between 1:4 and 1:7. The best staining is accomplished in from twenty to forty minutes.

This method, which has recently been adopted by Bignami and Bastianelli for the study of the crescents and the flagellate bodies, is superior to all the others so far published in the clearness of the image given and the special staining of the chromatin. Of late several modifications of these processes have been proposed for which better and more constant results are claimed, but for a description of which we must refer the reader to the special works upon the subject. Zettnow has recently proposed the alkalization of a solution of methylene blue by soda, which hastens the staining of the chromatin, and prevents the formation of precipitates. For this purpose he uses a one-per-cent. solution of the medicinal methylene blue of Höchst, 50 c.c., to which he adds 3 to 4 c.c. of a five-per-cent. solution of crystallized soda. To two parts of this solution he adds one part of a ten-per-cent. solution of bromeosin B. A. extra of Höchst. As a differentiating and decolorizing fluid he uses a solution of 2 gm. of methylene blue in 400 c.c. of water, with 1 c.c. of acetic acid. In from two to four seconds this solution extracts the blue from the hæmoglobin, and used twice for from three to five seconds it uniformly clears the preparation.

To study the malarial parasite in the viscera of the cadaver, we may use fresh preparations or dried and stained sections of tissues. The

parasites in the cadaver become very quickly altered, so that the preparations cannot be used for a minute study of their structure; the characteristic annular form of the young parasites, for instance, assuming a micrococciform appearance. Still the various parasitic forms remain for a long while, are distinctly recognizable, and can be stained, even in organs which are beginning to putrefy.

To find the parasites in splenic juice and in the bone marrow, we use preparations dried after Ehrlich's method and stained according to the method described for the staining of blood taken from the living subject. This method also gives most excellent results in the search for the parasite in the capillaries of the brain. A small portion of cerebral substance (preferably the cortex) is crushed between two cover-glasses and spread out, as in the case of the examination of sputum; it is then allowed to dry in the air and is fixed in equal parts of absolute alcohol and ether for about half an hour; it is then stained with hæmatoxylin and eosin. This method gives the clearest image of the capillaries and of the parasite-infected red corpuscles contained in them. In the preparation of sections Bignami uses a fixative agent composed of a one-per-cent. solution of corrosive sublimate in distilled water, to which are added 0.75 per cent. of sodium chloride and from one-half to one per cent. of acetic acid. The specimens remain in this liquid for from a quarter of an hour to an hour or several hours, according to their size, and are then passed through iodated alcohol to remove the precipitate of bichloride and then through alcohol. Excellent results are also obtained by fixation in formalin (five to ten per cent.) or in absolute alcohol. The best substances for staining are magenta red, hæmatoxylin, and methylene blue.

Description of the Malarial Parasites.

Nearly all authorities agree that there are several species of malarial parasites, but there is great difference of opinion as to the number of species which may be clearly distinguished, and as to the criteria which may serve to characterize them.

Three species of malarial parasites have characteristics which are so clearly recognizable that by a consensus of opinion they must be considered as natural species. These are: (1) Estivoautumnal parasites, (2) tertian parasites, and (3) quartan parasites.

Each of these corresponds to a determined clinical species of malarial infection (see the section on the classification of malarial fevers). The differences upon which these classifications are based are of various natures: in the first place, there are the morphological differences and those relating to the duration of the cycle of develop-

ment; in the second place, we have the clinical differences in the disease which each of these species produces in man; in the third place, the epidemiological differences, or those relating to the geographical distribution of each species and the various seasons in which each species is predominant.

While all agree in recognizing that the tertian and the quartan parasites are distinct, some hold that the so-called estivoautumnal parasites do not constitute a single species, but rather a group of distinct species.

Our own opinion upon this point is that the estivoautumnal species includes several varieties which are very closely related and to be distinguished from each other not so much by their morphological characters as by the mode of their pathogenic action upon man. For this reason we have divided them[24] into two varieties: (*a*) the one producing true quotidian fever, (*b*) the other causing the estivoautumnal or malignant tertian. We believe that other varieties very probably exist in these species, but as yet the data do not permit us to assert this as a fact.

A discussion of these disputed points must necessarily be preceded by a description of the parasitic species clearly to be distinguished as such. We begin with the estivoautumnal, because, although it is the one the biology of which, because of its complexity, was understood at a later date and after more assiduous labors than in the case of the tertian and the quartan, yet it possesses several phases of life so distinctly characteristic, even from a morphological standpoint (crescent stage), that a full comprehension of them will greatly facilitate the description of analogous phases in the other species. After giving this description, we will submit the various classifications proposed by different authors, and discuss the differential diagnosis between the species.

Estivoautumnal (Summer-Autumn) Parasites.

These are found in the groups of fevers which predominate in the summer and autumn in the malarial regions of temperate climates and in the tropical fevers properly so called.

The young forms of these parasites are represented by small protoplasmic bodies which are found either adherent to the surface of the red blood corpuscles, or in a sort of niche on their surface or edge (see Plate IV., Fig. 24) or else within their substance (*non-pigmented plasmodia* or *amœbæ* of Marchiafava and Celli). They may be immotile (discoid and annular forms) or endowed with lively amœboid movements.

The forms undergoing development are endoglobular, and we find in them very fine granules of black or brown pigment arranged with more or less regularity at the periphery of the parasitic bodies (*forms with pigment granules*); these also may be immotile (discoid or annular) or may move like small amœbæ.

In the more advanced stages of endoglobular life in which the parasites are preparing to multiply, the pigment at the periphery of the parasite shows a tendency to gather at one point at the centre or slightly eccentrically (*forms with blocks of pigment*) or in one block or in a thick mass of black granules. While the young non-pigmented forms and those in process of development are found circulating in the peripheral blood, the bodies with blocks of pigment are found accumulated in the vascular system of the viscera (spleen, bone marrow, brain in cases of pernicious fever, etc.), excepting in very grave infections, in which they may be found in the circulating blood. This distribution of the various parasitic forms in the vascular system constitutes one of the most important characteristics of the estivo-autumnal parasites as distinguished from the other species.

In the forms with blocks of pigment the parasitic body divides into a variable number of round or ovoid bodies, all alike and of the same size, which arrange themselves usually in a single or double wreath around the pigment. This segmentation which occurs within the red corpuscle, whose size the parasite does not attain even at its most advanced stage of growth, constitutes the mode of multiplication of the parasite in man (*fission* or *sporulation forms*). These forms, like those of the preceding stage, are found stationary in the vessels of the viscera.

The little bodies resulting from fission invade new red corpuscles and recommence the life cycle just described. These successive phases of existence constitute the *human cycle* of the parasite or, as it has also been called, the *pyretogenous cycle*, because of its intimate relation to the development and succession of the febrile attacks.

But not all the parasitic forms develop in just this way. In all cases, a certain number of young pigmented bodies continue to grow and become ovoid or spindle-shaped, while the pigment increases in amount and takes on the appearance of needles or little rods. When these fusiform or ovoid bodies by their increase in length have become longer than the diameter of the red blood corpuscle in which they have developed they usually become curved into a crescentic form (*body No. 1* of Laveran, *crescents*, *ovoid*, and *fusiform bodies*).

These bodies, which begin in man the life cycle which is continued outside of the human body, present certain important life phenomena which may be seen in ordinary fresh preparations if observation be

continued for some time. After ten minutes, or even more, we see that while certain of the crescents persist in their typical form, others become ovoid and then round. Then in the round bodies there begins an active movement on the part of the granules or rods of pigment, followed by the tumultuous thrusting forth of several filaments, usually four, which move like flagella. They usually become detached and continue to move with the greatest rapidity among the red corpuscles (*body No. 2* of Laveran, *flagellated body* of the majority of writers). The biological significance of these bodies cannot be understood except by following the later phases of development in the mid-intestine of the mosquito as we shall do later.

These forms (crescent phase) represent the beginning in man of a cycle of life which is continued and completed in the mosquito. Unlike the forms of the first cycle they have no pathogenic action in man and we might call them forms of the *anophelic cycle* because, up to the present time at least, their development has been seen only in some species of mosquito belonging to the genus *Anopheles*.

The intimate relationship between the successive phases of development of the parasite in its *human cycle* and the clinical course of the infection will be described in the section on Estivoautumnal Infection. Here we will describe the various forms of the parasites as regards their biological properties and structure.

Bodies of the Pyretogenous Cycle.

Young Non-Pigmented Parasites (Non-Pigmented Plasmodia of Marchiafava and Celli).

See Plate II., Figs. 1–9.

Seen in fresh preparations these occur as small whitish protoplasmic masses, possessing rapid amœboid movements, which take place at the ordinary temperature of the atmosphere (from July to October) and are quicker than those of the leucocytes at the same temperature. In a state of rest they are discoid in shape; from this form they pass to the most varied shapes, such as stars, crosses, and other odd forms, pushing out slender, diaphanous prolongations which oscillate in the substance of the red cell; they sometimes even ramify, and then become retracted while new ones are pushed out at other points of the periphery. After a while they become round again. At the ordinary summer temperature these movements may continue for from twenty to forty minutes, sometimes for as long as five hours. When they have ceased or are about to cease, they can be revived by using the warm table and by passing through it a current of water at

39° to 40° C. (102.2° to 104° F.). Sometimes the little body moves as if it were about to emerge from the corpuscle, but its pseudopodia never go beyond the limits of the red cell. When it stops moving it takes on a circular form, which is whiter at the periphery than in the centre (*discoid form*). Careful observation of one of these discoid forms often shows that the central part gradually becomes differentiated more and more from the periphery; little by little it loses its whitish aspect, and through it can be seen the hæmoglobin of the red corpuscle; the periphery on the other hand becomes more distinct and of a shining white, as if it were thicker. Thus is formed a distinct ring which looks as if it were printed on the red cell (*annular form*); by reason of its greater refractive power this is more prominent than the preceding form.

These small rings may be seen to change aspect; the cytoplasm which forms the ring spreads towards the centre which resumes its whitish diaphanous appearance, and it also spreads peripherally, the parasite thus becoming larger and of a more uniform appearance. It gradually returns to the discoid shape, which is larger than the ring from which it starts, and into which it may again become changed. The ring which has become discoid may again possess amœboid movements, but they are slower than before.

This succession of forms (amœboid, discoid, and annular) may be easily followed by examining one parasite only with a homogeneous one-twelfth immersion lens. It was by this means that these little bodies were seen and described by Marchiafava and Celli [12] who, having at first limited their investigation to primary cases of grave estival infection (which seemed to lend themselves most readily to the study of the malarial parasite), were led to attribute the greatest importance to these little bodies. In these cases, in fact, the above-mentioned bodies are those which chiefly attract the attention of the investigator, and among all the forms assumed by the malarial parasite in its development this is the one which by its special characteristic of motility gives the strongest impression of a living being.

By the description given of these young forms, we see that when in a state of rest (discoid form) they closely resemble discs, that is to say, they are so flattened that their lesser diameter corresponds to the thickness of the red blood cell. This can be observed when we see the little amœba endwise, or when turning upon itself, it is seen obliquely (which, however, occurs very rarely).

It is much more difficult to decide upon the true significance of the annular bodies. Marchiafava and Celli held that the so-called *rings*, which are so plainly seen as if printed on the red corpuscle, were merely amœboid bodies which, becoming very thin at the centre,

allowed the central part of the red corpuscle to show through them; by this thinning of the central portion and consequent thickening of the peripheral zone, there is formed a biconcave lens-shaped figure, somewhat resembling the corpuscle itself. In fact, if we observe one of these ring-shaped bodies when it presents itself endwise (which as we have said is rare, but still may occur), we have actually the impression of a biconcave lens. Antolisei, who thought that from the absence of analogous facts it was highly improbable a living organism could take on such an appearance, held that the annular forms were merely small amœbæ which had included in their substance a small particle of the red cell, as Osler and Councilman first suggested. We, taking into consideration the way in which we see a discoid form become annular during the microscopic examination, are unable to accept Antolisei's interpretation. Were it correct, we should see the amœboid parasite, when about to become annular, lengthen itself like a rod, then curve itself like a horseshoe, and finally unite the two pseudopodia after having included a portion of the red cell—in fact, the thing would have to occur as it does when an amœba or a leucocyte includes a foreign body; whereas, as we have stated above, it takes place in an entirely different way.

We have reason to believe that the annular form represents a parasite with a central vacuole, around which the substance of the amœboid body arranges itself in ring form. When treating of its structure as seen in stained preparations, we shall discuss the arguments by which this belief is sustained. For the present, we will merely note that as the vacuole is transparent it is easy to understand why in the examination of a fresh specimen the ring seems to be entirely empty at the centre, and seen endwise it has the appearance which Marchiafava and Celli compare to a biconcave lens.

We do not mean to deny that there are forms of the annular body which are nothing else than young parasites which have included a portion of the red corpuscles, as Osler, Councilmann, and Antolisei hold. But these must be distinguished from the typical annular forms, which, as can be seen from the description, are merely parasites whose protoplasm has contracted in ring shape around a diaphanous, very transparent substance, which constitutes the vacuole. This vacuole disappears in the bodies of the next phase when the stage of nuclear multiplication approaches. It also disappears (which is interesting to know) in the cadaver; indeed, in the cadaver we do not see annular bodies, but only as a rule immotile discoid or spherical micrococciform bodies. Annular bodies also disappear from malarial blood which has been strongly cinchonized. All this leads to the belief that we have to do with a vacuole which

is of great importance in the nutrition of the parasite in its young stage.

On the other hand, the forms which include a particle of the red corpuscle behave in an entirely different manner; first of all, whether the parasite be immotile or in motion, the included particle may always be seen and followed, while in the annular forms the vacuole becomes invisible so soon as the parasite alters its shape and puts out pseudopodia. Moreover, in the particle of included hæmoglobin we often see brown granules of melanin which have come from the transformation of the hæmoglobin, while nothing like this is ever seen in the central portion of the annular forms; but even before the transformation of hæmoglobin into melanin has begun, we can see that the included hæmoglobin is of a darker color than normal and somewhat resembles old brass. Finally, we may see plasmodia with fragments of included hæmoglobin in the cadaver and in strongly cinchonized blood, while the annular forms are not found in the blood under such circumstances.

The *structure* of these young forms as shown in preparations stained by methylene blue or by hæmotoxylin is the following: there is a very thin ring which is colored blue or violet, and which is deeper and thicker in one-half than in the other; the ring surrounds a space which takes the same stain as the red corpuscle, especially in its youngest stage, while in the centre of more developed forms the red corpuscle is of a paler appearance than it is outside of the ring; there is therefore in this form a very diaphanous portion of the parasitic body, which prevents a perfect appreciation of the color of the corpuscle. In the thickness of the stained ring we see one and not infrequently two or more very small granules of chromatin, which treated by Romanowski's method are clearly visible against the blue substance of the ring, being stained red or purplish red. Around the chromatin granules we do not see a clear halo or the constituent parts of a true nucleus, as we do see in part at least in the succeeding phases. We have said that the chromatin forms a part of the colored ring; in fact, in the majority of parasites it is intimately connected with it; in some it seems to project from the ring into the substance of the globule, and in others (but rarely) it is found in the centre and is not connected with the peripheral colored ring in any perceptible manner. In the cases in which the blood was fixed while the young parasites were in motion, we do not see them occurring as regular circles, but with deformities and prolongations, as they were sketched by Marchiafava and Celli as early as 1883.

The explanation which can be given of the appearance described above is the following: the little body of chromatin represents what

is rendered visible by our technical methods of a nuclear formation which is apparently very simple; the blue ring is the protoplasm of the parasite, which includes a nutritive vacuole (central transparent portion).

The aspect of these forms in preparations which are properly stained renders them clearly distinguishable from the irregular spots stained by the basic aniline colors which are seen within the red corpuscles—colored dots and spots the nature of which has been variously interpreted by different authorities. We must, however, give the points in the *differential diagnosis* of the young non-pigmented bodies seen in fresh preparations, because not infrequently those who are not expert in this kind of examination confuse the plasmodia with accidental vacuoles or with other alterations of the red blood cells.

Now in addition to ordinary vacuoles we not infrequently see in the red corpuscles portions completely deprived of hæmoglobin, which may be of various forms; some occur as hyaline rods, some as very small round bodies, as rods curved in a horseshoe shape, and even rods elongated into spindles with a point at the centre or nearer to one or the other extremity which is about the color of the red cell or a little darker; these last are the forms which may easily be mistaken for the rings. Finally we may see, but rarely, small shining white rings. All these forms, to whose resemblance to the young non-pigmented plasmodia Antolisei has also called attention, we have not infrequently seen in the blood of patients suffering from various diseases, but more especially from tuberculosis, typhoid fever, pneumonia, and suppurative fevers; as a rule they are few in number, but in some cases they are fairly abundant. One who is familiar with malarial blood has no difficulty in distinguishing these alterations of the corpuscles from young plasmodia; it is only necessary to see the special refraction of the hyaline bodies, which is much greater than that of the parasites. Confusion is impossible if we continue the observation of the suspected bodies, which may indeed (like the vacuoles) exhibit slow alterations of contour and even oscillate as if they were about to turn around on their own axis, but we never see any movements even faintly resembling amœboid movements. If we watch the succession of the forms which we have described, from the annular to the discoid and to the amœboid, we shall be very sure of our diagnosis of parasites. Staining shows that the little bodies in question have no characteristic structure.

Forms in Process of Development (*Plasmodia with Pigment Granules*).

See Plate II., Figs. 10–32.

The young parasites just described increase somewhat in size and begin to show a slight darkening around the contour, although no black pigment granules are discernible in this contour even on the most careful observation. We also see forms which have included a portion of the red corpuscle (clearly distinguishable from the annular forms) which while under observation become slowly modified in color and darkened. These changes precede the pigmented phase.

The latter is represented by parasites a quarter or a third the size of the red corpuscle, with very fine granules of pigment which are usually collected at the margin, but are sometimes scattered within the protoplasm of the parasite. We must also note that in many forms the pigment is only apparently marginal, because the peripheral granules at the border between the parasitic body and the substance of the red corpuscle are more clearly seen than the others. Many parasites at the same stage as the preceding are of much smaller size. The pigment granules are for the most part immotile, but they may also sometimes oscillate like the pigment of the parasites in ordinary tertian—especially in the large forms. These pigmented bodies may take on the same form as those of the preceding phase, or present an annular, discoid, and motile form. The discoid forms often have a crenated outline—the motile forms may take on strange shapes, such as the dendritic; the annular forms are smaller than these discoid and motile ones, as though they were contraction forms, and they are capable of returning easily to the motile condition.

Even in this phase we must distinguish the annular forms properly so called from the forms which contain one or more fragments of hæmoglobin. The differences are the same as those before described. The first have the appearance of a shining ring one-half of which is thicker than the other, in sickle form, with the centre showing the red corpuscle, of proper color or paler than normal; when they become motile again, they spread out, become more diaphanous, and in the central portion take on a color like that of the periphery of the parasite, but usually fainter. The parasites that hold a fragment of hæmoglobin may assume any shape without the fragment becoming invisible; this fragment is darker than the red corpuscle, and has a tendency to become brassy even when the rest of the globule is of normal appearance.

During this phase the amœboid movements of the parasites continue to be very active until the changes are initiated which lead to

multiplication by fission. As the parasites develop, the pigment granules which at first were almost imperceptibly small, become larger and tend to coalesce into three or four small masses which then take up an eccentric position; at this stage the parasite is more rarely of the dendritic form just mentioned, but usually becomes discoid. In this manner, by a diminution of motility and by an increase in size of the pigment granules and a tendency on their part to collect into groups the next stage is gradually reached.

As to the *structure*, the stained preparations show that it is the same as in the preceding stage. The parasites occur as rings, which are readily stained by hæmatoxylin, methylene blue, etc. The colored ring is not of uniform thickness, but usually falciform, this falciform appearance being even more distinct because of the increased size. The pigment is chiefly found at the periphery of the colored ring.

The chromatin globule which is larger than in the preceding stage is the same as to situation and other particulars. In preparations stained according to Romanowsky's method, this globule which is colored a violet red appears with great distinctness upon the blue ring of protoplasm. In the more developed forms we find that it is not homogeneous, but is composed of filaments and rods of chromatin; it is usually surrounded by a pale zone, which is as a rule not visible in the preceding stage.

The parasite therefore consists of a little mass of chromatin (the chromatin sometimes occurring in threads) surrounded by a halo of a pale substance, both together constituting a nucleus, and of protoplasm containing black granules, which is disposed in annular form around a vacuole. In the more advanced forms of development, and therefore nearer to the next stage of development, the vacuole is no longer to be found; in this case the parasite consists of a somewhat large nucleus with distinct outlines, in which is clearly seen the chromatin and the pale substance surrounding it (probably nuclear juice), but the membrane of which is not visible, and of protoplasm which is especially pigmented at the periphery.

Parasites in Process of Division (Bodies with Central or Eccentric Blocks of Pigment).

See Plate II, Figs. 33–42.

The chief characteristic of this stage is the division of the nucleus, which goes on until a variable number of very distinct nuclei have been formed; division of the protoplasm follows, with the formation of daughter forms (so-called spores).

Starting with the bodies of the preceding stage, we can follow them as they increase in size; the pigment also increases in the form of fine granules which tend to collect into larger granules, and finally in a block or clump of granules gathered at the centre of the parasitic body, or else situated eccentrically, or even at a point in the periphery of the body itself; these clumps may be two or three in number or exceptionally even more, and in this case each one is naturally smaller than the single block which is the usual form. When the pigment is collected into granules or little rods, these may be immotile or endowed with rapid oscillating movements.

The size of these bodies varies from a quarter to a half of that of a red blood cell—sometimes they are even larger, but the average size is about a third of the corpuscle. But there are some so much smaller (as we have seen in cases of pernicious fever) that two or three may be contained in one corpuscle and still leave a part of it unoccupied.

The *structure* of these little bodies is not seen in fresh preparations; they appear to be homogeneous and composed of protoplasm which is rather strongly refractive; in the more advanced stages of development we find at the periphery a series of shining dots which, as we shall see, indicate an advanced stage of the process of division.

When stained with hæmatoxylin in preparations dried according to Ehrlich's method and fixed in absolute alcohol and ether, these little bodies are found to consist of one part which is colored a deep purplish blue, usually at the periphery of the parasite, and of another very slightly stained and less extensive than the first, which we find to correspond with the pigment. In many of these bodies the little mass of chromatin cannot be made visible by this method, which circumstance has suggested to Bastianelli and Bignami and to Mannaberg that it is dissolved and mixed with the protoplasm. In a more advanced stage of development, one nearer to multiplication, we see towards the periphery of the stained portion of the protoplasm a variable number of minute bodies of a more intense blue color which represent the chromatin bodies of the future spores.

The results obtained by the hæmatoxylin method show therefore that there is a stage of development during which the chromatin cannot be demonstrated, and that this is followed by another in which the chromatin globules again become distinctly visible towards the periphery of the parasite, together with blocks of pigment.

The process of nuclear division can, however, be followed much more perfectly and without any interruptions by Romanowsky's method, which stains the chromatin a different color from the protoplasm.

In specimens thus prepared we find that the chromatin in every stage of development is clearly to be distinguished from the protoplasm, it being of a purplish red, while the latter is of a more or less deep blue. By this method, indeed, we find that some of the bodies with blocks of pigment (usually the smallest) are composed of a peripheral portion stained blue (protoplasm) and of a central or sub-central part formed of granules or filaments stained red (chromatin) surrounded by a pale substance which remains almost or altogether unstained (nuclear juice). By the side of these are seen other bodies with blocks (usually larger than the preceding) in which are two or three clumps of chromatin, each one surrounded by a zone of pale substance. These small masses, seen under a high power, appear to be dentate at the periphery, and to be composed of filaments so closely packed that in some specimens they cannot be clearly distinguished. In other specimens each of these little collections of chromatin filaments is seen to divide into two distinct masses, which are at first very closely placed together, but later separate, and are then surrounded by a pallid zone, and arranged with more or less regularity in the protoplasm. Thus by a successive series of divisions of the nuclear protoplasm, we have the formation of a varying number of little round or ovoid bodies of chromatin which are readily stained and are compact in appearance, that is to say, without recognizable structure and apparently homogeneous. At this point occurs the division of the body with the block of pigment into daughter bodies.

During the process of successive divisions of the chromatin there is evidently a notable increase in its amount; in each successive phase the amount is greater, and that found in a body with a block of pigment in which the daughter bodies are already formed, is very great in comparison with that of the chromatin in the original solitary nucleus.

The Multiplication Forms (Fission or Sporulation Forms).

See Plate II., Figs. 43–48.

When, as described in the preceding stage, the nuclear chromatin has successively divided into a varying number of ovoid or round bodies, a portion of protoplasm arranges itself about each one which then also divides, and thus fission is complete; a very small portion of protoplasm with melanin remains non-utilized and forms the residuum of segmentation. These forms which have undergone fission appear in fresh specimens like an accumulation of round or ovoid bodies gathered around a block of melanin and occupying from a third to a half of the red blood corpuscle in which they are situated; there

are forms both smaller and larger than this. In each of the daughter bodies we see for the most part a small shining spot, as in the spores of quartan parasites.

In stained preparations we can make an exact observation of the number and of the structure of the spores. Their number is somewhat variable; some small fission bodies with only six, eight, or ten spores take up not more than a quarter of the red corpuscle; other larger ones, also endoglobular, have spores which form two rows around the block of pigment and are as many as thirty or even more in number. From the study of several cases of pernicious fever in which the sporulation forms were very numerous, it would seem that fourteen to sixteen is the average number of spores.

Each spore is composed of a little chromatin body which is very strongly stained (nucleiform body or nucleolus) surrounded by a thin stratum of protoplasm; the form is round or ovoid. In only a few sporulating forms do we succeed in detecting a pale zone around the nucleolus, as in the spores of the tertian or the quartan; as a rule this zone is not visible.

When fission has occurred, perhaps as a result of swelling of the pale substance which is situated between the individual spores and constitutes a part of the residuum of segmentation, the red corpuscle bursts open, and the liberated spores disperse in the plasma. We can see this exit of the spores from the corpuscle very easily under the microscope. The freed spores adhere to new red corpuscles; in grave cases we have often seen two, three, or more spores clinging to a corpuscle. These are easily distinguished from young parasites, (1) because they have a determined and constant form which is round or ovoid; (2) because they do not possess amœboid movements; and (3) because of the absence of a vacuole. The transformation of the so-called spore into a young amœba occurs with the appearance of amœboid movements and of the vacuole, which it is to be assumed plays an important part in the processes of nutrition. Upon the formation of the vacuole the young amœba assumes in stained preparations the typical annular appearance which differentiates it from the young bodies resulting from fission.

If bodies resulting from the segmentation of an adult parasite do not possess the above-described structure, then in our opinion they cannot be regarded as spores, but as degenerative products. In a fresh specimen we cannot with certainty recognize an isolated spore, but must have recourse to appropriate staining. Spores in a fresh specimen can be recognized only when they are grouped in a characteristic manner. Not all parasites with pigment blocks give rise to multiplication forms such as we have described. Some swell up, are

very feebly stained by aniline colors, become vacuolated, and disintegrate into small, unequal, pale masses. This process, of which every stage can be seen, is evidently of a degenerative nature.

Duration of the Life Cycle.—The cycle just described, from the non-pigmented forms to sporulation, with the invasion of new red corpuscles, occupies, according to our researches, about forty-eight hours, giving the *tertian* estivoautumnal fever. We have stated as our belief that there is a variety of the same species, which completes its cycle in about twenty-four hours, giving a *quotidian* fever, but this point, which is still in dispute, we will take up later. The duration of the *human* life cycle of this parasite is neither so regular nor so constant as is that of the quartan for instance, but has certain oscillations and irregularities; these are, however, not sufficient to justify the opinion of some authorities who refuse to recognize any law in the duration of the development of this parasite, or any type in the fevers produced by it. As a study of the life cycle demonstrates that the irregularities in its duration occur only within determined limits, so a study of the febrile curve shows that a rhythm in the fundamental febrile type is clearly to be recognized.

Forms which Begin in Man the Life Cycle which is Completed in the Mosquito.

These are represented by the semilunar stage (*crescents, ovoid and fusiform bodies, round bodies of crescent origin, and flagellated bodies*) regarding the origin of which there has been much discussion of late, as there has also concerning the terminal phases, the biological significance, and the structure.

The *crescents* have the exact shape indicated by their name: they are cylindrical cells thinner at the two extremities than in the centre, transparent and colorless, a little longer than the diameter of the red cell (8 to 10 μ), and in breadth one-third the same diameter (2 to 3 μ), curved in the form of a crescent; in the central portion are grains or needle-shaped rods of melanin. The outline of the parasitic body is indicated by a single very fine line; in some cases this line may be double. On the concave side a very fine line appears to unite the two ends of the crescent.

The fusiform bodies have the shape indicated by their name; their size is about that of the preceding forms which they resemble in all their characteristics, except that they are not curved upon themselves. As a rule their ends are very slender; the pigment may be gathered at the centre, and may be irregularly scattered in the parasitic body or arranged along the long axis of the spindle. We have found this form most frequently in grave infections and pernicious fevers.

Ovoid bodies are shorter and thicker than the crescents and have their pigment irregularly scattered or more often collected in the central portion and arranged in the form of a wreath.

All these forms are devoid of amœboid movement, and even the pigment is immotile. As a rule, however, when they are carefully examined under the microscope, we see some changes in their shape; thus we may see a crescent become ovoid and then become transformed into a round body, with a wreath of pigment, whose later stages of development we shall study presently.

All are endoglobular, as was recognized by Marchiafava and Celli, who regarded the very fine line which appears to unite the two curved ends of the crescents as the faint outline of the red blood cell. The corpuscle which contains them is always very pale, and sometimes, while the blood cell is pale, the crescent form has a slight hæmoglobin tint, as though it had attracted to it the coloring matter of the corpuscle, forming of it a sort of cuticle. We have also seen two crescents within the same blood corpuscle, the curved portions being face to face. Laveran held that the crescents were simply adherent to the globules, and that this adhesion was merely accidental, but a simple microscopical examination and above all a study of their development shows that they are endoglobular. A good dry lens (for instance, No. 5 or No. 7 Hartnack objective) will show them, without the necessity of an immersion lens.

The study of the *structure* of the crescents has given rise to many discussions. As a result of our most recent researches we hold that a crescent body is formed of a vesicular nucleus in the centre of the parasite, around which are arranged needles or rods of pigment; and of cytoplasm which surrounds the nucleus and is more abundant in the portions corresponding to the greatest diameter of the fusiform body; there is no membrane.

As to the presence of a membrane, opinions have, up to the last few years, been very contradictory. Laveran, regarding the crescents as cystic bodies, thought there was a membrane, but this was disputed later, as a result of their microscopical observations, by Marchiafava and Celli (1887). Celli and Guarnieri still later (1889) interpreted the double outline, which is seen in some but not all crescents, as indicating the existence of a rather thick membrane. The same theory is held by Mannaberg, who, as we shall see, regarded the crescents as syzygies resulting from the fusion of young parasites and therefore provided with a membrane. It is true that the double outline is not seen in all, but only in a few crescents, but it remains when these change into spherical bodies. It is true also that this double outline is seen only in fresh specimens, and not in stained

preparations, but Mannaberg rests his belief in the presence of a membrane chiefly upon the phenomena that occur during the process of flagellation (see below). But the same phenomena, we observe, may also be seen in the flagellation of the tertian bodies—*i.e.*, rapid undulatory movements of the contour of the parasitic body and active movements of the pigment, phenomena which give the impression as of the existence of bodies moving rapidly within a cyst—and yet according to the consensus of opinion these have no membrane. The theory that the crescents have a membrane has evidently been held by many, not as a result of the direct observation, but in consequence of the preconceived notion that the crescents are resistant bodies—a preconception resulting from the long persistence of these bodies in the patient's blood in spite of the administration of quinine, which appears to have no action upon them.

The correct interpretation of the double outline seen in some of these bodies has, in our opinion, been given by Antolisei, who considers it to be a species of hæmoglobin cuticle formed from the red corpuscle, within which the crescent has developed. As to the round adult bodies of crescent origin, they have a sort of adventitious envelope, formed from the peripheral portion of the red cell which has gradually been more or less completely invaded by the parasite. The existence of these pseudocysts explains the impression received by an observer of the change of a body of crescent origin into a flagellated body—the impression, namely, of a sudden liberation of the flagella from a restricted space in which they have been enclosed.

As to the nucleus, Celli and Guarnieri, studying the structure of these bodies according to their method (methylene blue dissolved in ascitic fluid), noticed that at the centre of the crescent and close to the clump of pigment was a small round body which was often stained blue, and which, according to these authorities, was similar to the little bodies which in the coccidia are regarded as nuclei. Later Grassi and Feletti described in the crescents a vesicular nucleus which for the most part was round, situated in the middle of the crescent body, and provided with a chromatin body (nucleoliform node of these authorities) which might be large or small and might be seen to divide into two or four; sometimes, however, even when the method described was employed, it was not discernible.

Very evidently the discussion in regard to the structure of the crescent body could not be brought to an end until we were in possession of some method capable of demonstrating the constant presence of chromatin in all crescent bodies. By Mannaberg's method we frequently do not succeed in demonstrating its presence; by the hæmatoxylin method of Bastianelli and Bignami, in the majority of

cases we are unable to see the so-called nucleolus, a fact which caused Bastianelli and Bignami to suppose that this formation was absent in bodies in the semilunar phase, especially as by the use of the same method they were always successful in finding the chromatin in the other phases of life of the estivoautumnal parasite. More recently Ziemann, adopting Romanowsky's method, has vigorously upheld the theory that crescents as a rule do not possess nuclear chromatin, and he even believes that he has thus provided a morphological basis for the opinion that the crescents are sterile bodies.

It is a singular thing that it was precisely in consequence of the adoption of Romanowsky's method that Bignami and Bastianelli recently modified their early views upon the subject. They found that if preparations of blood were made in the usual way and kept in a moist chamber to prevent rapid drying, and then stained by Romanowsky's method, nuclear chromatin was very clearly seen in all the crescents, and in the ovoid and round bodies of crescent origin, occurring in the form of violet-red granules, while the protoplasm was of a more or less intense blue color.

The contradictory results first obtained by Ziemann using the same method are probably due to the fact that, working with preparations dried immediately, after mounting he did not see the chromatin because it was hidden by the granules of melanin surrounding it. In preparations kept in the moist chamber, however, as the nucleus and body of the crescent swell and the pigment needles disperse, the chromatin becomes visible, swells somewhat, and is stained by Romanowsky's method in the typical way. The difficulty in demonstrating the chromatin in the crescent bodies by means of other methods, which, as we have seen, give inconstant results, is apparently because this substance is much less easily stained in the crescents than is the chromatin body in the young parasitic forms.

Origin and Development of the Crescents.—The researches carried on in Rome have for a long time shown that the bodies of the semilunar stage are developed from the estivoautumnal parasites, of which they represent a constant life phase.

The young parasitic forms from which the crescents originate are distinguishable from other forms of this species of parasite, even when they are less than a quarter of the size of a red blood cell. They occur as small, round, ovoid, or spindle-shaped bodies, which when seen in a fresh specimen appear to be quite homogeneous and to contain a greater amount of black pigment than do the bodies of equal size of the preceding cycle; the pigment, moreover, is in the form of little rods or of somewhat large granules, and is either irregularly disseminated in the body of the parasite, or collected chiefly

towards the periphery. These forms are not motile, they always occupy the lateral portion of the red corpuscle, and in their development always tend to adapt their convex surface to the edge of the corpuscle itself.

As the development proceeds, even the bodies which were originally round tend to take on a long ovoid or rather spindle form, so long as the distance between the poles of the ovoid or the spindle does not exceed the diameter of the red corpuscle; when it does, the body either keeps the same shape or it becomes curved and forms the true crescent.

The structure of the young forms is essentially the same as that of the adult bodies. When stained by Romanowsky's method we see a cytoplasm which is colored blue more deeply at the periphery than towards the centre, and a nucleus which in the young forms is rather large in proportion to the amount of cytoplasm; the latter, however, during the further development increases in volume much more than does the nucleus. The nuclear chromatin is stained a purplish red, and is usually in the form of threads or rods, sometimes of granules; in some cases it collects at the centre of the parasite and is surrounded by a pale zone; in other cases the granules or threads of chromatin are disseminated more or less irregularly in the parasitic body; but this latter appearance may possibly be due to some accidental variation in the preparation of the specimen. Therefore as regards the character and disposition of the chromatin also the young bodies of the crescent stage are distinguished from the parasites of equal size belonging to the first (pyretogenous) cycle.

These various forms of development of the crescent body are rarely found in the peripheral blood; in this we usually find only the adult forms, the young forms occurring there only in grave infections with a large number of parasites in circulation, and not always then. In the spleen we may find crescent bodies, and follow their endoglobular development, even when they are not found in blood taken from the finger. But in pernicious fevers we have often found a large number of young crescent bodies in the bone marrow, when the same forms were very scarce or altogether absent from the blood in the other organs. These facts suggest that the bone marrow is the chief if not the exclusive seat of the formation of the crescents. The accumulation of young crescents in the bone marrow was noted several years ago by Bignami and Bastianelli; autopsies in cases of grave infection afterwards performed by us have yielded us results so in harmony with this point of view that we are able to exclude absolutely the possibility of this accumulation being an accidental occurrence.

An opinion altogether different from the one which we have expressed as to the origin of the crescent bodies has been advanced by Mannaberg, who believes that they are syzygies derived from the fusion of several young parasites. This hypothesis is based upon what he believes to be an established fact, that the crescents possess a membrane, and upon the theory that the young amœboid forms, of which several are found within one red cell, end by merging into one body; the syzygy, according to this theory, is capable of multiplying later, and by its multiplication gives rise to the relapses. As to the mode of multiplication Mannaberg maintains some reserve, but he holds it to be not impossible that there is a segmentation along the minor axis.

Not infrequently several young parasites are seen in the same red corpuscle; we have counted up to six or seven, and when they are very close together they may appear to be intimately adherent. Starting from these figures, Mannaberg takes pains to describe and to draw all the forms which might be considered as transitional between the young flattened parasites and the young crescents.

In opposition to this theory of Mannaberg, we would advance the following considerations and facts: in the first place we cannot hold it to have been conclusively demonstrated that the young parasites collected within one red corpuscle become merged together; on the contrary, they follow their own development, as shown by the fact that we often find several forms in process of multiplication within the same corpuscle, or various amœboid forms in process of development; in the second place, the crescent forms, as we have already said, do not possess a membrane of their own, but a species of adventitious membrane formed from the red corpuscle (it is to be noted that the formation of a syzygium is usually followed by encystment); in the third place we would recall the fact that we have direct evidence of the entire developmental series of the crescent bodies in the bone marrow, a demonstration which can naturally not be made by one who has not at hand the proper materials for study.

As to the later stages of development of the supposed syzygies, we shall see that the theory of multiplication by segmentation in relation to the relapses is not sustained by the data furnished by recent investigations in regard to the development and the biological significance of these parasitic forms.

In spite of the many and varying opinions expressed as to the origin and the significance of the crescents, all authorities agree that the crescents, as first pointed out by Marchiafava and Celli, proceed from the small amœboid parasites, which multiply by sporulation in the manner described (parasites of estivoautumnal fever).

Grassi and Feletti alone hold that the crescents are derived from parasites which do not sporulate with the described succession of forms (bodies with blocks of pigment, etc.) but form crescents only. They made of this a special genus, the "*Laverania*," and to the species which occurs in man they gave the name, *Laverania malariæ*, in contradistinction to the parasites causing grave fevers, to which they gave the name of *Hæmamœba præcox*. Therefore the various forms which we have described as constituting two life cycles of the same parasite (pyretogenous cycle and crescent cycle) according to these authorities constitute two species of two different genera.

This view was opposed by Bignami and Bastianelli who, making systematic investigations in cases of estival fever by means of frequent punctures of the spleen, demonstrated that in all cases of this group which are studied for a sufficiently long time without medical treatment, we can always follow the development of the parasites on the one hand up to the body with central pigment and its fission, and on the other up to the young endoglobular crescents. This proves that the crescents are merely one phase in the life of the estivoautumnal parasite. It is not necessary to add that most of the recent researches, as we shall see later, by demonstrating the biological significance of the crescent bodies, absolutely exclude the theory once held by Grassi and Feletti.

Final Modifications of the Crescents.—We would note in the first place that in preparations, especially those kept for some time under observation, we often notice alterations in the crescent bodies which must be held to be degenerative. For the most part the parasitic body divides into numerous masses of unequal size, hyaline and of simple outline, which gradually disappear within fifteen to twenty minutes or a little more, as if they were dissolved in the serum. By watching this process of disaggregation we acquire the certainty that the crescents have no membrane, because if there were one, it ought to become apparent during this disintegration. We may also see the process of vacuolization of the crescent bodies as well as of the ovoid and round ones.

But in addition to these degenerative alterations upon which we have always insisted, and which (as we may now assert with absolute certainty) constitute the final phase of the crescents if they do not reach the surroundings adapted to their further development, that is to say the mid-intestine of some species of mosquito, several writers have described a segmentation (sporulation) of the semilunar bodies; and even those who acknowledge that they have never seen this up to the present time, hold that there must necessarily be a process of multiplication.

Thus Grassi and Feletti claim to have seen two forms of segmentation of the crescents, one of fission scarcely begun, the other of completed fission, similar to what is seen in the segmentation of the parasites of the regular fevers. Mannaberg describes a transverse segmentation which usually occurs in the middle of the parasitic body, dividing the crescent into two equal parts. Canalis describes the sporulation of round bodies of semilunar origin, and even gives a drawing of it. Golgi held that in the crescents there was a "process of internal differentiation," which led to the formation and to the emission of young parasites which invade new red corpuscles, whence occur renewed febrile attacks. For the sake of brevity we omit the opinions of many others who have taken up the argument.

Those who held that there was a multiplication of the crescent bodies without being able to demonstrate it, based their belief upon the fact that the crescents persist in the blood during the apyretic interval separating a group of febrile paroxysms from the relapse, and thought that the latter could be explained only by a process of sporulation of the crescents themselves. Those who have described and pictured this sporulation were evidently led into error by their preconceived notion, and mistook a degenerative process of disintegration for sporulation; this was the case with Canalis, for instance, who, as Bignami notes, described a sporulation in which, to judge from his own designs, the nucleus took no part at all.

We ourselves, basing our belief upon numerous and careful observations, have always held that the crescents do not multiply in human blood. This affirmation of ours, of which recent experimentation has shown the truth, was based (1) upon the fact that even under the best conditions of research we never succeed in finding a fission form of crescents, which could with certainty be held to be a sporulation; and (2) upon the fact demonstrated by the researches of Bignami and Bastianelli, that the relapses of the fever are not in relation to the development of the crescent bodies.

The only developmental forms of the crescent bodies which can be studied in preparations of the blood are the so-called flagellated bodies.

Pseudo-Flagellata and Motile Filaments.—When under the microscope we observe a preparation of blood containing crescents, we find that some of these are motionless, or only slowly change into ovoid or round bodies; others, however, so soon as they have become round in shape, exhibit lively movements of their pigment granules, and suddenly shoot out filaments which are endowed with great motility —that is to say, they turn into flagellated forms. These are never seen immediately after the specimens are prepared, but some time

after the blood has been taken from the circulation, and for this reason it is supposed that flagellation is a phenomenon which does not occur so long as the parasite remains in the human body.

The so-called flagellated body is composed of a pigmented hyaline body, which is smaller than a red blood corpuscle; the filaments (flagella) start from the periphery, either singly at various points, or all together from one point, and sometimes forming a bundle which separates into two or three prolongations. The flagella are four or five times as long as the diameter of a red blood cell, sometimes longer, and are usually pointed at their free extremity, although they may be bulbous, or they may present swellings along their continuity. Their motion is continuous or may be interrupted by pauses. Sometimes they meet and rub each other, as do the feet of a fly; sometimes they whip the neighboring red blood cells, push them about and change their shape; then they become detached and move off rapidly in the plasma, scattering the red corpuscles which they meet. But sometimes their motion gradually stops before they become detached, and then on careful observation we find them after a while motionless and adherent to the pigmented body. During the movement of the filaments the pigmented granules of the parasitic body usually remain at the centre, but they may be carried to the periphery, and even penetrate into the prolongations (which then appear to be canalized) where they exhibit rapid movements either in the direction of the free ends or backward into the pigmented body again. The movements of the pigment granules within the pigmented body sometimes cease during the motion of the filaments, but again they may continue for hours even after the filaments have stopped moving and have become detached. It is extremely rare in a fresh specimen to see a flagellated body in which the including red corpuscle is distinguishable, that is to say, a round body in which there are flagella which can be clearly seen to be endoglobular, such as Marchiafava and Celli have seen and made a drawing of; but in properly stained preparations we sometimes see around the parasite the shadowy outline of the red corpuscle, showing that the flagellated body is within a completely decolorized corpuscle.

If we add a drop of distilled water to a specimen under the microscope, in which there are bodies with flagella in active motion, the movements cease, and all trace of the prolongations is lost, while the pigmented corpuscle remains distinctly visible. A physiological sodium-chloride solution, however, does not in the least affect either the form or the movements of the flagella.

In addition to this series of phenomena, we may perceive the emission of small, round, hyaline bodies which become detached

from the edges of many of the various forms of the crescent stage—that is to say, as well from the crescents as from the round or flagellated bodies. They first project from the edges, and then detach themselves and either move away from the parent body or remain close to it. As many as two, three, or even five little bodies may thus make their exit from one body of the crescent stage. This process, to which we shall return later, has by some been described as a *gemmation*.

But not all the actively motile bodies of the crescent stage end in the putting forth of flagella. Marchiafava and Celli have also described special bodies with an undulatory movement of the contour; these bodies may be seen to revolve in one direction and then in another, while their peripheral portion is the seat of the most rapid undulatory motions; at the centre they have a pigmented nucleus which is either motionless, or seems to swing in correspondence with the peripheral oscillations. The movement of these bodies lasts from about twenty to forty minutes, when it slackens, becomes intermittent, and finally stops. All this may be seen in the ordinary fresh preparations. But to acquire an exact idea of the structure of these bodies, and of the flagellata especially, we must examine preparations stained according to Romanowsky's method.

By this method Sakharoff studied the flagellata of a hæmatozoon found in the blood of young crows taken from their nest in malarial regions; he describes the so-called flagella as chromosomes which have come out of the nucleus of the parasitic body, and flagellation as a process of perverted karyokinesis, which is performed in a confused manner—that is to say, as a phenomenon of degeneration.

Bignami and Bastianelli have recently made use of the same method in the study of the structure of the flagellated bodies derived from the crescents, using preparations in which the blood was spread in the usual manner on the cover-glass, and which were kept in a moist chamber for ten or twenty minutes or more, then rapidly dried and fixed in absolute alcohol for twenty-five or thirty minutes. Under these circumstances many of the crescents became flagellated; indeed, Marshall, Ross, and Manson have demonstrated that a certain amount of moisture and of exposure to the air favor this phenomenon. Such crescents as do not become flagellated usually undergo certain alterations which will be described presently.

In these preparations the crescentic and the round bodies usually become swollen to some extent; even the nuclear chromatin swells and takes on the form of granules, or blocks, or filaments. Occasionally some minute chromatin masses are seen to have made their

exit from the parasitic body and to be adherent to its periphery (the so-called *bud* to which we have alluded).

The flagellata are found to be composed of a pigmented body, which takes a light blue stain, and in which the chromatin is divided into blocks arranged along the periphery; from these peripheral chromosomes project filaments of chromatin which, surrounded by a very thin layer of protoplasm, constitute the individual flagella. In cases in which we see an isolated motile filament (flagellum), one which is completely formed, we find that the chromatin tends to be massed at the centre, the extremities being formed of protoplasm; the chromatin may also occur in the form of a series of granules or rods instead of filaments. In stained preparations in which we are able to ascertain with accuracy the number of the flagella, we usually find four for each body, rarely more.

Not all the flagellata, however, possess the morphological characters above described; there are some in which the filaments do not contain any chromatin but are composed of protoplasm alone, and others in which there may be one or two filaments provided with chromatin, the others being formed of protoplasm. Such forms are to be considered as incomplete; that is to say, as flagellata whose development has been interrupted by desiccation. In fact, these forms as well as some others of irregular aspect, the description of which we shall omit here for the sake of brevity, are not found at all when the process of flagellation is completed, not in a moist chamber, but in the place where it naturally occurs, that is to say, in the mid-intestine of the *Anopheles*.

Crescents which do not become flagellated, when kept in the moist chamber under the same conditions as the others swell to some extent and exhibit characteristics differing somewhat from those of the bodies which give origin to filaments—that is to say, their cytoplasm takes on a deeper blue stain, the chromatin is in smaller amount, and is gathered in granules and rods in the nucleus which is either central or subcentral and surrounded by a wreath of pigment; but as a rule a certain number of granules of chromatin are seen to have made their exit from the nucleus and to be adherent to the periphery of the cytoplasm. These granules constitute the so-called buds, which, as we have said, can also be seen in fresh preparations, and may likewise be found at the periphery of the flagellated bodies.

We must therefore recognize two classes of crescent bodies, those which become flagellated, and others which do not and which differ from the first in some minor morphological details.

Biological Significance of the Crescents and of the Bodies Derived from Them.—The most varied opinions have been held in regard to

this matter. Some of these we can dismiss at once, taking note only of the facts demonstrated regarding structure. Thus we cannot admit that they are cystic bodies, as Laveran and after him other writers, as Mannaberg, believed, since it has now been proved that they have no membrane. Nor is it to be admitted that they are resistant spores, as was believed by Councilman, who was struck with the resistance opposed by these forms to quinine, and this because of what has been learned in regard to their biological properties and because of the facts already described in relation to their ultimate development. It is also easy to dismiss the idea that they are bodies capable of multiplying in human blood, as was held by Golgi, Antolisei, Canalis, Grassi and Feletti, and others who reasoned by analogy rather than from well-proved facts.

We have always opposed to these views the theory propounded by Bignami and accepted by many other authorities, namely, that crescents are sterile bodies. Bignami[26] considered the crescents to be forms of "divergent and interrupted development of the estivoautumnal parasite"; he said *forms of divergent development* because, following the life phases of the parasites, he thought he observed that, at a certain point in their growth, while some were preparing to multiply others deviated from this course and without multiplying continued to increase in size until they formed typical crescents; he added *forms of interrupted development* because at a given moment these forms, without multiplying, degenerated in various ways and disappeared in the blood. This same theory was later taken up by Bignami and Bastianelli,[21] who undertook the systematic study of peripheral and of splenic blood from patients suffering from primary estivoautumnal fevers, with the view of ascertaining the time in the course of the disease at which the first crescents appeared, how long they persisted in the blood, what was their relation to the relapses, etc. And since it was ascertained by these researches that no crescents were ever seen to multiply, and that they could not be held to be the cause of relapses, these writers held that they were the *sterile forms* of the summer-autumn parasite. They took up the subject again in a work published in 1894[31] in which they endeavored to explain the sterility of these bodies by the theory that the crescents have the same biological significance as the forms belonging to several other parasites which complete their life cycle outside of the organism in which they are found. This analogy they expressed in the following terms. "It is a well-known fact that two cycles of development have been demonstrated in several endocellular parasites belonging to the group of the coccidia. One cycle of development is completed exclusively during the parasitic life. But after the parasite

has lived as such during a series of generations, there begins a second life cycle, represented by forms which can terminate their development only in the surrounding atmosphere or in the tissues of some other animal. Should these forms of the second cycle not make their exit from the body of the animal in which they started they remain sterile, and after a while degenerate and die." From these words we see that Bignami and Bastianelli held that the crescents were sterile forms in man and for man, and we may affirm even now that this view, if the observation be limited to what occurs in human blood, as was the case until recently, is the pure and simple statement of a fact which has now been definitively proved.

The most recent observations show that in truth the crescents reached the tissues of another animal (that is to say the mid-intestine of a mosquito) and there complete their life cycle, as will be described later. Thus has the problem of the biological significance of these forms been fully solved.

What is the significance of the so-called flagellata? Upon this point also opinions have been various and uncertain. It is well known that Laveran considered his "motile filaments" to be the final and perfected phase of the malarial parasite.

Marchiafava and Celli held that the motile filaments should be regarded as protoplasmic prolongations of the pigmented bodies, having the significance of flagella, and they regarded the flagellated bodies as representing a later development of the pigmented plasmodia. Grassi and Feletti, on the other hand, held that the so-called flagellata were merely a product of the degeneration and destruction of the adult parasites. Labbé also considered them to be forms of death, not found in the living organism, but outside of it only, a product of the physicochemical action which modifies the plasma and the corpuscles which are withdrawn from the blood-vessels. Of course the results of recent researches regarding the structure of these bodies has completely demolished these theories.

Mannaberg opposed this view; if these were forms of death, he said, there would be no explanation of the fact that they are seen only in a limited number of parasites. He expressed the suspicion that the flagellated bodies represented the first principle of an extra-human phase of life, which dies from the lack of a soil adapted to their needs.

Manson has recently advanced a theory which in part resembles the foregoing. He holds that the flagella are pre-formed within the crescents and the round bodies (as Laveran held), from which they make their exit when both crescents and round bodies are outside of the human organism. He has also endeavored to demonstrate, by

staining with carbolized fuchsin, the presence of the filaments preformed within a delicate cyst. According to this theory, the flagellum is a special form of spore, which is developed only in the outer air, "in the interest of the extracorporeal life of the parasite"; the seat of this ulterior development being the body of a suctorial insect, specifically the mosquito. This theory has not been sustained by later researches. A study of the structure has in fact shown that the flagella are not pre-formed in the crescents and the round bodies; within these we find the filaments of chromatin which go to make part of the flagella, but not to form them in their entirety. Finally, the researches of Ross upon the proteosoma of birds, and those carried on in Rome upon the malarial parasite in man have shown that the pigmented bodies and not the non-pigmented flagella are developed in the mosquito.

In these later days there is increasing belief in the theory, which we uphold, that the crescents and the flagellata are sexual forms of the malarial parasite, and that a reproductive act (in which the flagellum represents the male element and an adult crescent the female cell) gives rise to the new being which begins its existence in the tissues of the mosquito.

The facts upon which this idea is based are found first of all in the sexual phenomena which occur in various sporozoa. Simond was the first to propound the theory that the flagellata of malaria are sexual forms. He studied the life phases of *coccidium oviforme*, of *cariophagus salamandræ*, etc., and found in these parasites two cycles: (*a*) a cycle which he called *asporulate*, which is completed in the host, and gives rise to the formation of falciform corpuscles, *merozoites;* and (*b*) a *sporulate cycle*, represented by encysted forms, which is completed outside of the host, insuring the life of the coccidium in its new surroundings. Now this second cycle begins by an act of fecundation, the male element being represented by an adult form in which the nuclear chromatin is divided into a large number of filaments which separate and go to the periphery of the parasitic body, from which they emerge and remain for a while adherent to its edges like a horse's mane and then detach themselves, being surrounded by a thin layer of protoplasm. In fresh specimens this process occurs with so rapid a movement that the bodies appear to be flagellated. The filaments are spermatozoa which fecundate the young coccidia, and these then begin the sporulation cycle, or the cycle by which is assured the conservation of the species outside of the host, and the possibility of fresh infections. These facts established, the author expresses it as his theory that the polymitus of Danilewsky and the flagellata of malaria have the same signifi-

cance and the same functions as the pseudo-flagellata of the coccidia (1897).

Schaudinn and Siedlecki, when studying the development of the *Adelea ovata* (Schneid.) and of the *Eimeria Schneideri* (Butschli), were able to follow in the most complete manner the development of the sexual forms and fecundation, and describe the accompanying nuclear modifications. Applying zoological nomenclature to the coccidia, they call the sexual forms in general *gametes*, the female elements *macrogametes*, the cells producing the male element *microgametocytes*, and the male elements *microgametes*. Siedlecki later (1898) studied similar phenomena in another coccidium (Klossia).

Inspired by Simond's studies, MacCallum endeavored to ascertain whether sexual forms and phenomena of copulation were to be found in some hæmatozoa, and was fortunate enough to witness under the microscope the act of fecundation in the Halteridium of birds. He divides the adult forms of the Halteridium into granular forms and forms of a homogeneous hyaline aspect—the latter only becoming flagellated. A flagellum penetrates into a granular adult form, which after fecundation becomes changed into a motile body resembling the "vermiculus" of Danilewsky. Later, studying a case of estivoautumnal infection in man, in which there were many crescent forms, MacCallum twice witnessed the penetration of a flagellum into a round body of crescent origin with wreath-shaped pigment.

We ourselves have not witnessed the penetration of a flagellum into a spherical body of human malaria, perhaps because we have not had cases with a large number of crescents at our disposal. In spite of this, we admit the theory of fecundation as that most consonant with the latest knowledge in regard to the biology of sporozoa. It would seem indeed from the most recent researches that the sexual phenomena are constant in these beings, and that the sexual act precedes the formation of the encysted forms which begin the cycle that is continued outside of the body.

Reasoning from analogy, therefore, it seems probable that the forms which pass from man to the mosquito are sexual forms, and that an act of fecundation initiates the new life cycle in the middle intestine of the insect. In substantiation of this theory are the recent researches of Bastianelli and Bignami, according to which, by the use of Romanowsky's staining process, we find two kinds of crescent bodies, differing in the amount of their chromatin, which is greater in the forms that become flagellated, and in the staining of the cytoplasm, which is of a much deeper blue in the non-flagellated forms.

Following Schaudinn and Siedlecki, who have studied the sexual forms of the sporozoa, we will call the crescent bodies *gametes*, the

bodies which become flagellated (male elements) *microgametocytes*, the individual flagella *microgametes*, and the bodies which do not become flagellated (female elements) *macrogametes*.

With these phenomena whose seat is naturally not the outer world (where until lately they were chiefly observed), but the middle intestine of some species of mosquito, begins the new life cycle which is completed within its tissues. This will be described below.

General Morphological and Biological Properties of the Estivoautumnal Parasites.—It would appear from the description just given that all these parasitic forms consist of a *nuclear formation*, whose constituent parts are not seen with equal clearness in all the various stages of their existence, and of *cytoplasm*.

In the very young forms all that we see of the nuclear formation is the chromatin globule, to which various writers have given different names. Mannaberg and others call it the nucleolus, Grassi and Feletti the *nucleoliform node*, while we prefer, as a rule, to designate it as the *small body of chromatin* in order to avoid cytological discussion. Similar small bodies have been described in many lower organisms, as for instance in the coccidia; some writers, following Labbé, call them *karyosomata*, others, such as Rhumbler, call them *Binnenkörper* in order to adopt an indifferent name. In some coccidia these small inner bodies (Binnenkörper) can be distinguished from the nuclear chromatin which is arranged in threads and granules, and we can see what becomes of each during the process of division, as Schaudinn and Siedlecki did in the case of the *Adelea ovata*. It would appear as a result of these researches that the Binnenkörper are not the same as the nucleoli of the cells of higher organisms.

In the summer-autumn malarial parasite the technical methods at our disposal do not permit of a clear distinction between the nucleolus and the nuclear chromatin.

In bodies which are in the course of development, we see around this chromatin body a light zone which is supposed to be nuclear juice and which is not apparent in the very young forms.

As to the nuclear membrane, which, according to some authorities, circumscribes the clear zone, we can demonstrate it only in the adult bodies of the second cycle (crescents), but by none of the methods in use can we find it with any certainty in the young and in the developing forms, and even less in those undergoing multiplication. We can only assume the probability of its presence, first by the distinctness with which the nuclear formation is seen to be separated from the cytoplasm, and secondly, by analogy with what is known as to similar organisms whose structure can be more easily studied because of their greater size.

The *protoplasm* (cytoplasm), as we have said, becomes pigmented at the periphery, but in the crescents we find the pigment gathered in the innermost portion of the cytoplasm, immediately surrounding the nuclear formation. We have described in it a vacuole, which is almost constant in the very young forms and which disappears in the course of their development; in our opinion its presence is the cause of the annular forms assumed by the plasmodia in fresh preparations, and of the appearance which these same take on in preparations stained according to Romanowsky's method, in which they are seen as thin blue rings, in which the chromatin body is seen of a purplish-red color, while the central portion (vacuole) is colorless or pale.

The *phenomena of motion*, as we have said, are very lively in the young forms, and diminish by degrees to cease entirely during the stage of multiplication. These movements concern the protoplasm, but it is probable that the nucleus also has amœboid properties. In the crescent cycle we have no movements except such as accompany the formation and the extrusion of the motile filaments.

Multiplication occurs by the successive division of the nuclear chromatin (rudimentary form of karyokinesis), within an adventitious cyst formed by the red corpuscle. The little bodies resulting from the division are not provided with a membrane; they have been called spores or gymnospores, and the process of their formation *sporulation*, because these expressions are in current use among naturalists to indicate multiplication by segmentation in similar organisms. It is to be remembered, however, that the meaning is not at all the same as that of spore in bacteriology. In fact, these spores of the malarial parasite have none of the biological properties of the enduring spores of bacteria, that is to say, they are not endowed with special powers of resistance, and as regards their structure, as has been said, they do not differ in any essential points from the young plasmodia. Antolisei held that the spores of malarial parasites were provided with a membrane (clamidospores), but this view has not been confirmed by more recent observers. Bastianelli and Bignami have suggested that some spores which were born naked might, under special conditions, acquire a membrane and lose their staining capacity; but this is a theory put forth in explanation of a few cases in which, during a period of latent infection, it has not been possible to find even in the spleen any parasites to the presence of which could be referred the occurrence of a late relapse; it is a theory, however, which up to the present time has not been proved.

The estivoautumnal parasites develop like the others within the red blood corpuscles. Lavearn thought that the parasites were free in the blood or simply adherent to the red cells, but later Marchia-

fava and Celli held that the plasmodia were endoglobular, this view being based upon the fact that their pseudopodia never went beyond the boundary of the red cell; furthermore, the amœboid bodies are seen as if floating in the protoplasm of the corpuscles, becoming less visible, or, as it were, submerged, and then reappearing, one or two prolongations first becoming visible and then the whole body. They also noted that sometimes the plasmodia were only partially enclosed in the red corpuscles as though about to leave or entering them.

Mannaberg, while still believing in the endoglobular situation of the adult bodies, has recently expressed a doubt as to whether the young plasmodia (non-pigmented plasmodia of Marchiafava and Celli) were within the red cell. According to Mannaberg a direct proof that some forms are endoglobular is found in the observation of the spherical bodies of the crescent stage, or the large tertian bodies (see below) at the moment when they are leaving the red corpuscle, for it is impossible to doubt that they make their exit by the bursting open of the corpuscle itself. But the small, non-pigmented forms, according to this observer, long remain simply adherent to the globules. He says that it is very often difficult to determine whether a parasite is within a corpuscle, or simply adherent to it, or, as it were, pressed into its surface; in the latter case it might be that the pseudopodia were unable to go beyond the limits of the corpuscle (as noted by Marchiafava and Celli) simply by the fact of their viscosity which might prevent them from becoming detached from the cell. He holds that they are in a sort of depression in the corpuscle, and says that by means of oblique illumination and an open diaphragm which allows us to examine the body in relief, we can make out such a depression with distinct edges on the surface of the corpuscle.

In spite of this, we maintain that the greater number of parasites, even the young non-pigmented ones, are endoglobular. First of all, we would call attention to the fact that if we once admit the endoglobular situation of the developing and the adult forms, the question loses all its interest—there merely remains for us to ascertain at what period of their development the young parasites enter into the substance of the red corpuscle. In our opinion, the theory that the majority of the young forms are endoglobular is demonstrated by the fact, to which we have already called attention, that by following their motions we see them apparently become submerged in the substance of the corpuscle, and then emerge from it again. Furthermore, in the cadaver, in which the parasites have lost their amœboid qualities, we sometimes see them moving *in toto*, with a floating motion, within the corpuscle, as though the contents of the red blood cell were liquefying. The impression received is that the globule is

transformed into a little bladder full of fluid in which the parasite is seen to oscillate if the corpuscle is moved.

Young parasites, as we have said, sometimes seem to be embedded or pressed into the surface of the corpuscle, but forms which are at the beginning of the pigmented stage are scarcely ever seen in this position, but are always endoglobular. This shows that the young plasmodia, after remaining for a short time adherent to the red corpuscle, penetrate within its substance; nor does their development progress until they have done so.

But although the fact that the seat of development of the parasite is endoglobular is established, it does not follow that there is an equal distribution of parasites throughout the vascular system. The mechanical conditions of the circulation differ in the various viscera, and moreover as each viscus modifies in a special manner the blood which circulates in it, the conditions of development for the parasite must differ in the various organs. It is an interesting fact that the parasites in their several life phases, while still endoglobular, may have a predilection for certain situations. Thus the young forms of the pyretogenous cycle circulate in the blood, while the adult forms, bodies undergoing multiplication, and fission forms as a rule remain stationary in the internal viscera. This may be partly due to the alterations produced in the corpuscle as the parasite develops, but it is partly and perhaps chiefly owing to a special biological property of the summer-autumn parasite. The bodies of the crescent group behave differently, the young ones being found only within the vessels of the viscera, and are chiefly, if not exclusively, formed in the bone marrow, while the adult forms enter the general circulation. We must see in these facts a phenomenon of adaptation; the adult crescents indeed are the bodies which, when taken in with the blood by a mosquito, continue their development in the intestine of this insect, and therefore, if they circulate in the blood for several days, they have just so much more chance of completing their life cycle.

For a discussion of the *pathogenic action* of the estivoautumnal parasite upon man, and the relation of its development to the clinical forms of the fever, the reader is referred to the section on the Symptomatology of Estivoautumnal Fever.

Parasites which Complete Their Entire Life Cycle without Becoming Pigmented.

In 1885 Marchiafava and Celli, who had already previously described the fission of the pigmented bodies, suggesting the theory that this represented the multiplication of these plasmodia, called

attention to the fact that this fission of the plasmodia may occur even before the red cells in which they have developed are entirely destroyed; moreover, they described small endoglobular fission forms that possessed not a trace of pigment.

In some cases of pernicious fever they found upon staining sections of the cerebral cortex with vesuvin that the capillaries were overfilled with red corpuscles, many of which contained parasites in all stages of development, all of which were non-pigmented; these were young discoid forms, forms in process of division, and forms already divided into a small clump of ovoid bodies all of equal size, sometimes arranged in rosette form, and staining like the so-called spores of the malarial parasites.

They judged from these observations that there are malarial parasites which may complete their whole cycle of development without becoming pigmented; it is possible therefore that there may be malarial infection without melanæmia.

These data were confirmed later by Bignami, who studied the parasites in a large number of cases of pernicious fever, and then by others (as Marchoux, who studied the malaria of Senegal). Later still in various animals endoglobular parasites were found which completed their whole life cycle without producing pigment (Smith and Kilborne in the Texas fever of cattle, Dionisi in bats, etc.).

Limiting ourselves to what is observed in pernicious fevers, we must first premise that for these researches we can make use only of fresh specimens, or of those which have been preserved in alcohol for a short time only. We know, in fact, that the pigment may gradually disappear altogether from brains kept for a long time in alcohol, so that when we find in them non-pigmented fission forms, we cannot be sure that the loss of pigment is not artificial. And in regard to observations made during life, we would add that we can never be certain of the existence of an infection without melanæmia, until repeated punctures of the spleen have demonstrated the complete absence of melanin.

Fission forms without pigment in the cases observed by us have for the most part been composed of eight to ten spores, rarely more; the young forms are in structure exactly like those already described; the forms in process of development differ from the ordinary summer-autumn parasite only in the absence of black pigment.

Do these forms represent a definite parasitic species, or are they estivoautumnal parasites, which have multiplied prematurely before the formation of pigment?

As in these later years we have not had the opportunity of making further researches upon this subject (in this region such cases being

extremely rare), we leave the question to be settled by future investigations. Some authorities, in spite of not having made researches themselves, but basing their opinion upon the observations made by Marchiafava and Celli, hold to the first opinion; thus Mannaberg speaks of a quotidian due to a parasite which does not produce pigment. In justification of our attitude of reserve, we call attention to the fact that in the cases in which we found non-pigmented fission forms, there were always pigmented parasites as well. It is also singular that while in the vessels of an organ, as, for instance, the brain, all the parasites in every stage of development might be non-pigmented, so that we had the complete cycle under observation, in the spleen in the same cases we found the usual fission forms with blocks of pigment.

Up to the present time, therefore, we have no example of a well-studied case in man, in which all the parasites in the vessels of all the viscera are without pigment—in other words, of a case which might be considered as a pure culture of the non-pigmented parasites.

Nevertheless, although we prefer to suspend judgment as to the question of species, we believe that these forms may be considered as related to the estivoautumnal parasites, in the first place because we have always found them in company with parasitic forms that could be diagnosed with absolute certainty as estivoautumnal, and secondly, because up to the present time they have been found only in pernicious or in tropical fevers.

Tertian Parasites.

These parasites, like the preceding, possess two life cycles, one of which is completed in man, while the other begins in man and is completed in some species of mosquito. The phases of development of this parasite, in relation to the regular clinical evolution of the ordinary tertian, were described by Golgi in 1889. The forms of the second cycle were first described by Celli and Antolisei; later Bignami and Bastianelli proved their analogy with the crescent forms, in spite of their different appearance, but only lately has their biological significance been understood.

Forms of the Pyretogenous Cycle.

See Plates I., Figs. 15–33, and IV., Figs. 10–22.

The parasites complete in man a life cycle which is in intimate relation to the successive febrile attacks, and the *duration* of this cycle, from the youngest forms to sporulation, is about two days;

that is to say, it is equal to the time which elapses between the beginning of one attack and that of the next attack.

During the course of its development this parasite becomes larger than a normal red blood corpuscle, that is to say, much larger than an adult estivoautumnal parasite, from which it differs not only in size but in appearance, in the nature of the pigment, of the sporulation, etc. The difference in pathogenic action between the two species is well known, and is described in the section on Symptomatology. The intimate structure, on the other hand, is essentially the same.

The young non-pigmented forms are so similar to those of the estivoautumnal parasite that it is very difficult to distinguish them under the microscope. They are usually a little larger, however, and less opaque. They are very motile and go through the usual changes in form, passing from the discoid to the annular form, and from this to the discoid again and to the amœboid. The forms in motion send out slender prolongations (pseudopodia) which may ramify in every direction, sometimes reaching the periphery of the red blood corpuscle, but not going beyond it; then they retract, and other similar pseudopodia project from other parts of the parasitic body. The most varied and strange forms imaginable result from this process.

While the similar non-pigmented phase in the summer-autumn species is of long duration, in the tertian parasites pigmentation occurs with rapidity. The pigment in the initial forms is scanty, and in very fine granules, and has a tendency to accumulate at the extremity of the pseudopodia. The structure of the young forms is like that already described in the estivoautumnal parasites; in preparations stained according to Romanowsky's method, they are seen to be formed of cytoplasm which is stained blue, against which is seen the chromatin body stained purplish-red, round or ovoid in shape, and larger than is the similar body in the first stage of development of the estival parasite. Around it we see a slender pale zone which separates the chromatin body from the cytoplasm; this is constant in these forms, but we do not always find it in the estival parasites, except at a little more advanced stage.

In the parasites which are free in the plasma, and in many endoglobular ones, we see only the above-described parts. But in other endoglobular parasites we may find the characteristic ring, that is to say, a blue ring which is, as a rule, thicker in one-half than in the other, enclosing a space which is of about the color of the red cell, or a trifle lighter. At one point of the periphery of the ring we find the nuclear formation already described, that is to say, the chromatin body surrounded by a pale zone. In these forms it is

easy to convince ourselves that we have not to do with a larger vesicular nucleus occupying the whole centre of the ring, but with a vacuole from which the nucleus embedded in the cytoplasm can be clearly distinguished by the limiting outline of the clear zone.

If the parasite be fixed during amœboid movements we may see the most varied forms. The round or roundish vacuole is usually found near the nucleus, and from the surrounding ring of protoplasm we see simple or ramified prolongations projecting, which are sometimes very long. Other non-vacuolated forms may have the shape of a horseshoe, or of a slender filament of cytoplasm curved upon itself in various ways, the nuclear body being situated near one of the ends of the filament, or even in the extremity itself.

In forms of more advanced development the special characteristics of the tertian parasite are more in evidence. The increase in size is very marked, so much so that in the first twenty-four hours the parasite may take up from one-half to two-thirds of the red corpuscle. This increase in volume is due chiefly to the growth of the cytoplasm, the nucleus not increasing proportionally.

In a fresh preparation we see that the parasite has acquired more definite outlines; it contains many granules of melanin, and possesses lively amœboid movements, which cause it to assume curious shapes within the corpuscle. Even when the movements of the cytoplasm are not shown by marked changes in the outline, we often see the pigment granules change place, sometimes slowly, but usually with great rapidity, looking very like the darting of flies. This motion is not trembling in character, neither has it the regularity of a Brownian movement; it may be more or less rapid like the plasmatic current, to which we believe it is to be attributed.

One of the most striking facts is that the parasite-containing red corpuscles are markedly larger and paler than normal; in fact, one of the most characteristic properties of the tertian parasite is the rapidity with which the infected red cell becomes decolorized and swollen.

The development of the parasitic body continues during the second twenty-four hours until it is about two-thirds to four-fifths the diameter of the enclosing corpuscle, which is usually the limit of its growth (adult body). In this last period of development it maintains the characteristics already described, with the exception that the amœboid movements are a little less rapid, for which reason it is apt to maintain a more or less rounded form, and does not assume the bizarre shapes seen in the younger stages.

The structure is the same as has already been described. We find, however, that the chromatin is a trifle less deeply stained than

in the very young forms, and, according to Romanowsky, it appears to be composed of very fine filaments and points which latter probably represent the cross section of the filaments. The cytoplasm, stained blue, is found to have increased proportionally more than the nucleus, and is markedly pigmented. The pigment naturally does not invade the clear zone which surrounds the nuclear chromatin, and which belongs to the nucleus (nuclear juice). In the forms which are as large as half the red corpuscle we often see a vacuole in the cytoplasm which may be near the nucleus or at the periphery; there may even be two or three vacuoles. The form of the vacuole is not always circular, but often irregular, and not infrequently prolongations from the cytoplasm may be seen within it; these are evidently pseudopodia that were surprised and fixed during motion. We have already stated that the protoplasm is stained blue, but the coloration is not always uniform, this probably being due to the difference in thickness of its various parts caused by the amœboid movements.

In the round and but slightly motile adult forms the chromatinic part of the nucleus is seen more clearly than in the preceding phases to possess a fibrillary appearance.

When this stage of development has been reached those changes begin which lead to sporulation, which is completed within the corpuscle after about forty-eight hours, and, as in the case of the summer-autumn parasite, coincides with the onset of a febrile attack.

In fresh preparations we see scarcely anything of the interior changes which precede fission; we see only the result, that is to say, the pigmented body in process of dividing, or already divided, into daughter bodies.

The forms seen in fresh preparations may vary, so much so that Golgi recognizes several methods of segmentation in the tertian parasite. The most frequent occurrence is to see adult pigmented bodies, in which the pigment is more or less entirely collected in the centre; around this pigment mass the parasitic body divides into a varying number of little spheres (about fifteen to twenty) which all together form a round mass. They do not essentially differ from those forms in which the pigment, instead of remaining at the centre in one mass, is found at the periphery, or is divided into two or three small clumps, or is even irregularly disseminated between the individual daughter bodies. A description with drawings of this form of segmentation may be found in the article entitled "Nuove Ricerche," etc., by Marchiafava and Celli.[12]

In other parasitic bodies the segmentation, according to Golgi, may occur in a very different way, namely, by the formation of figures

resembling sunflowers. Golgi describes this process as follows: When the pigment is gathered at the centre of the parasitic body, the peripheral portion of the latter appears to separate itself from the pigmented centre in the form of a ring. In this ring radiating striæ very soon appear, which at first are scarcely visible, but gradually become more and more marked, and which subdivide the ring into numerous portions composed of a whitish substance; these subdivisions gradually become individualized, so to speak, acquire definite outlines, and form so many little spheres which become detached from each other, and finally arrange themselves in the form of a wreath around a central pigmented disc. Golgi regards this as the form of segmentation most characteristic of the tertian parasite, and the one differing most from the fission process of the other parasitic species.

Finally, Golgi somewhat doubtfully indicates a third method of segmentation seen in the free bodies. In these bodies we occasionally observe that "the pigment, instead of being massed at the centre as usual, gradually becomes arranged in a zone more or less close to the periphery, this occurring in such a way as to determine a somewhat distinct line of separation between the part occupied by pigment and that which is free from it. The latter becomes exceedingly transparent, sometimes appearing like a vacuole, within which may be seen one, or more rarely several, spheres similar to those which result from segmentation."

Golgi has good reason to express himself with a little hesitation in describing this process as a segmentation; as a matter of fact, later studies on the structure have shown that this is not a process of multiplication but of degeneration, which occurs in the large pigmented bodies free in the plasma. At this point we must pause to state that the various changes described above which may be witnessed in fresh specimens in the fission forms occur with great rapidity at the onset of the attack, and represent only the latest phases of fission. During the microscopical examination all that we are able to witness is the more or less rapid separation of the daughter bodies from each other; but the various phenomena leading to the formation of the individual parasites cannot be followed in blood taken from the circulation—that is to say, in the ordinary preparations.

An examination of stained preparations, especially those made after Romanowsky's method, demonstrates that the gradual passage of the uninucleated adult body to a completely segmented form occurs in successive stages with marked slowness, the process sometimes taking more than twelve hours. This is fundamentally the

same process as that described for the estival parasites, but it can be more clearly seen because of the greater size of the tertian parasites.

We have already said that in the adult forms the nuclear chromatin presents a less uniform and compact appearance than in the young forms. When the stage of division approaches, the filaments or rods, which give a denticulated appearance to the chromatin mass, tend to separate, leaving clear spaces between them, so that the nucleus becomes two or three times larger than in the young forms. At the same time, the pale zone surrounding the chromatin becomes thinner and less easily distinguished than before from the surrounding protoplasm. The chromatin now divides into two masses, which sometimes take on the shape of semicircles, whose concave surfaces face each other, and are then transformed into more or less compact masses, surrounded by a narrow clear zone. We thus have two new nuclei, somewhat smaller than the original nucleus. From this, by a similar process of division, are formed other nuclei, which having a tendency to separate from each other arrange themselves towards the periphery of the cytoplasm. With each act of division the clear zone surrounding the chromatin becomes less visible, but after the formation of the new nucleus it again becomes more distinct.

The pigment, which never invades this clear zone, is pushed away by the successive divisions of the nucleus, and gathers in one or more clumps at the periphery or at the centre of the parasite.

By these successive divisions are formed pigmented bodies with two, four, six, or more nuclei. In preparations made about six hours previous to a febrile attack (which coincides with complete segmentation) we have seen pigmented bodies with about eight nuclei, more rarely ten or twelve. By successive divisions there are formed from sixteen to twenty nuclei, which is the average number of spores to which a tertian body gives rise.

The formation of daughter nuclei being completed, a portion of the cytoplasm condenses around each, becoming separated from the surrounding parts; thus is formed the daughter body (spore or gymnospore) which therefore is composed of a small mass of strongly stainable chromatin, surrounded by a narrow clear zone and by a ring of protoplasm which is of a deep blue color. As a rule the nucleus is excentrically situated in reference to this cytoplasm. A portion of the cytoplasm which is less deeply stained and which contains granules or needles of black pigment, remains unused and is called the residuum of segmentation.

At this point the red corpuscle, which in fresh specimens has become almost invisible from the gradual consumption of the hæmoglobin, bursts open, possibly by reason of the swelling of that part

of the protoplasm which represents the residua of segmentation, and the *gymnospores* become free. This may be seen to occur under the microscope.

In describing the process of fission, we have given the results obtained by the method of Romanowsky, who applied it to the tertian parasites (1891), and who was followed by Ziemann and others. The contradictory results obtained by other observers are due to the diversity of the technical methods adopted. Mannaberg, by staining with hæmatoxylin, was not able to observe the successive divisions of the chromatin, for which reason he believed that the chromatin (nucleolus), in forms about to divide, came out from the nucleus and mixed with the cytoplasm; therefore the stage in which the parasite is preparing to sporulate he considers to be characterized by the disappearance of the nucleolus (nuclear chromatin, nucleoliform node). This disappearance, admitted also by Bastianelli and Bignami in the case of the summer parasite, does not really exist. The error probably arose from the fact that there is a phase in the life of the parasite in which the nuclear chromatin cannot be distinguished by the depth of the staining (hæmatoxylin method) from the cytoplasm, and this is precisely the stage in which the chromatin undergoes the successive divisions. A method like that of Romanowsky, which gives a special color (purplish-red) to the chromatin and another (blue) to the cytoplasm, permits us, as we have seen, to follow the whole process.

As to the nature of the division process, Romanowsky holds that it is a species of karyokinesis, he having observed a fibrillary structure of the chromatin portion of the nucleus, which becomes accentuated at the time of the division.

Forms which Begin in Man the Cycle which is Completed in the Mosquito.

In addition to the form of the cycle described, in which it is easy to follow all the phases up to reproduction, others were long since described which, up to within the last few months, we have scarcely known how to interpret.

For a long time observers had perceived that in tertian blood, in addition to the pigmented bodies which, when they reached the adult stage, underwent the modifications described up to sporulation, there were others which, although they grew even larger than the first, did not become segmented, but underwent other transformations—that is to say, they in part became vacuolated, in part flagellated, and in part fell a prey to other degenerative processes.

These bodies were accurately described by Celli and by Antolisei, who held them to be forms of degeneration. Later Bignami and Bastianelli showed their analogy to the crescent bodies, and held that they possess the same significance here as do the latter in the estivo-autumnal species of parasite.

In their adult form they are seen, in fresh preparations, as round or somewhat elongated bodies, sometimes with a long diameter twice the size of that of an ordinary red blood corpuscle; the red cell containing them is of course enlarged and pale, sometimes even entirely decolorized. The protoplasm contains a large amount of black or brick-red pigment in most active motion, and sometimes there may be found a somewhat large hyaline vesicle, usually excentrically situated and evidently representing the nucleus. This is perhaps the only form of the tertian parasite in which the nucleus is visible in a fresh specimen without any staining; and as this nucleus has a distinctly vesicular appearance, and a decided outline which the pigment never goes beyond in spite of its mobility, it is likely that there is a nuclear membrane which is not visible in the stained parasites.

If we examine one of these bodies for some time, we often notice that at one particular point a small transparent sphere is formed and around it a vacuole, then near to the first one other spherules and vacuoles appear, until the whole parasite has been converted into a mass of globules of varying size, between which are disposed the pigment granules, whose oscillatory movements cease entirely. Sometimes while this vacuolization and progressive splitting of the parasitic body into hyaline spheres is taking place, a part of the cytoplasm projects beyond the red blood cell and here the oscillations of the pigment continue until this portion also is broken up into hyaline masses.

It was evident to Celli and Antolisei that this process of disaggregation and vacuolization indicates the death of the adult parasitic body. The description given by some writers of reproduction with vacuolization of the tertian parasites, a reproduction whose various phases can be followed by microscopical examination, corresponds perfectly to the phases of this degenerative change. That it is not a multiplication is demonstrated by the examination of preparations stained by various methods, which permit us to establish the fact that sporulation always occurs in the characteristic manner described above.

While, as we have said, some of these bodies are seen to become vacuolated and to divide into small transparent masses of unequal size, or to present both phenomena at once, others during the micro-

scopical examination suddenly exhibit a confused movement of the cytoplasm and the pigment contained in it, and then become converted into flagellated bodies, in precisely the same way as the round bodies belonging to the crescent stage become changed into flagellated bodies.

Small hyaline buds are sometimes seen coming from the pigmented masses which give rise to the flagella. And when the movement of the flagella ceases and they have become detached and removed from the pigmented body, a sort of clot of pigmented protoplasm remains behind and then divides into a variable number of transparent spherules; after a while the movement in the pigment stops, and all becomes quiet.

In preparations stained by Romanowsky's method we find that all the pigmented bodies, those which become flagellated as well as those which do not, are composed of an abundant amount of cytoplasm which is stained blue, and of a vesicular nucleus which contains threads or granules or rods of chromatin surrounded by a clear zone.

What is the significance of these bodies? When they remain in man they end in degenerative processes, and are taken up by the phagocytes, as occurs to the bodies of the crescent group. In fact, in the examination of stained specimens we find a few which contain very little chromatin or none at all (sterile bodies, as they are called by some). But when, in their adult stage, they are taken in with the patient's blood by the mosquito, they develop in the intestine of the latter, provided that it belong to the right species. Their biological significance therefore is identical with that of the crescent forms, and like these they are to be considered as *gametes*.

What is their origin? It has not been possible to follow their development as completely as that of the crescents, still Bastianelli and Bignami have observed that in preparations made after Romanowsky's method could be seen parasitic forms about half the size of the adult bodies described, which are distinguished from the forms of the first cycle multiplying in man by the nature of the nuclear chromatin. Their chromatin is arranged in threads and rods, sometimes forming a sort of reticulum, and neither so compact nor so deeply stained as in the forms capable of sporulating. These bodies are probably forms of gametes in process of development. It has not yet been possible to ascertain by direct examination whether the youngest forms are to be found in the bone marrow, as in the case of the young crescents.

We have stated that in our opinion these large pigmented bodies represent the gametes of the tertian parasites. The reasons for this belief are the same which we have given in the case of the crescent

forms—*i.e.*, reasons based upon morphological studies and upon analogy. It follows that these parasitic forms, like the crescents, should be divided into two classes—those which do not become flagellated (macrogametes) and those that do (microgametocytes). The characteristic differences between the first and the second can be shown in preparations of tertian blood kept in a moist chamber for fifteen or twenty minutes, then dried and stained by Romanowsky's method.

In the first we find the nucleus to be somewhat swollen and situated at the periphery of the protoplasm. In the second (microgametocytes) the nucleus is in the centre of the parasite, and contains a larger amount of chromatin (five or six times as much as in the macrogametes), which is gathered in apparently a single mass at the centre of the nucleus, or as deeply stained intertwined threads. From these bodies arise the flagella (microgametes) by a process which, to judge from the transitional forms, may be described as follows: The chromatin divides into a number of small masses which are carried to the periphery, each mass then becoming transformed into a filament which projects from the parasitic body, and is surrounded by a thin layer of protoplasm. All the chromatin is used in the formation of the microgamete.

These tertian pseudoflagellata differ somewhat from those of crescent origin which we have described; in their entirety they are larger, but the principal difference concerns the number of the flagella, which is usually greater, they as a rule containing six.

In the same way occurs the formation of the microgametes in their normal surroundings, the middle intestine of *Anopheles*, in which, as everything tends to prove, the fecundation of a macrogamete by the entrance of a flagellum takes place.

But we shall follow below (p. 75 *et seq.*) the further development of these bodies which are already found at an adult stage in the blood of patients with tertian fever a few days after the onset of the infection. In fact, as Bastianelli and Bignami have found, if a patient suffering from primary tertian infection be stung a few days after the beginning of the disease, the tertian parasites will already be found in a condition to develop in the mosquito.

Quartan Parasites.

In these parasites also two life cycles are to be distinguished, one being completed in man, the other begun in man and completed in certain mosquitos. The bodies of the first cycle are well known from Golgi's description (*Archivio per le Scienze mediche*, 1886);

those of the second cycle are not so generally known as the corresponding ones of the estivoautumnal and tertian parasites.

Forms of the Pyretogenous Cycle.

See Plate I., Figs. 1–14.

This life cycle develops in a period of three days; that is to say, in the interval between two typical quartan attacks, and is intimately and regularly related to the occurrence of these attacks; in fact, the stage of multiplication of the parasites coincides with the onset of a febrile attack (Golgi).

In fresh preparations made during a quartan attack, and during the two days of apyrexia between this and the next attack, we may easily follow the whole development of the parasite up to sporulation. No phase of life escapes the observer, because the development occurs in the circulating blood; while in the case of the estivoautumnal parasites, as we have seen, the adult and multiplying forms accumulate in the internal viscera, and even the tertian parasites show a certain tendency to accumulate at the same stage in the vascular area of the spleen, although not to such an extent that their entire development cannot be followed in the peripheral blood.

The *young parasites* occur as small endoglobular amœboid bodies without pigment, exactly similar to the tertian bodies, but less motile and less transparent. They appear in the blood during the febrile attack, and rapidly become pigmented, so much so that on the first day of apyrexia we find in the blood endoglobular pigmented parasites, about one-fifth or even one-quarter the size of the red cell, and endowed with torpid movements, as shown by the slow change in shape of their outlines. The red cells containing them are normal in size and appearance. During the whole period of apyrexia the parasites slowly increase in size, preserving the same appearance; their movements become gradually slower and slower, so that they do not tend to assume the irregular and grotesque shapes taken by the tertian parasites, but remain more or less round. As the parasite grows, the pigment becomes more abundant, and occurs in black granules, which are notably larger than in the tertian parasites, and usually non-motile. The red cell preserves its normal size, or, if at all modified, tends to be somewhat smaller; its substance is gradually replaced by the parasitic body which is developing within it, but around the latter there persists, up to the period of complete development, a sort of ring of a substance colored by hæmoglobin—indeed, the hæmoglobin coloration of the residual portion of the red cell may be even darker than normal.

The *adult forms* are round, pigmented bodies, almost as large as a red blood cell, which have invaded nearly the whole of the containing corpuscle of which only a slender zone around the parasite still persists. Along with these forms we see others in which there is apparently no trace left of the red corpuscle, but closer examination will show a very thin involucrum, which represents the peripheral residuum of the invaded cell.

These bodies, which have attained to the maximum growth of quartan parasites, are found in the blood from eight to twelve hours before the febrile attack, whose onset coincides with the end of their life cycle. We can see in them the first indications of the internal changes which lead to fission (sporulation).

Some of the phases of segmentation may also be followed in fresh specimens in the course of the last eight to twelve hours, and are the final stages of the process; on the other hand, as we shall see, in preparations properly stained, we find that nuclear division begins first in the adult pigmented bodies with irregularly disseminated pigment.

The following is what we witness in fresh preparations: In the adult forms the pigment tends gradually to accumulate at the centre, where from the beginning we find the pigment irregularly arranged in striæ or trabeculæ, or sometimes in striæ radiating from the centre to the periphery of the parasitic body; then the pigment which forms the striæ gradually gathers towards the centre, forming a globular mass with well-marked outlines. At the same time the parasitic body shows a line of division which little by little becomes more distinctly visible. The final result of all this is the formation of from nine to twelve pyriform or ovoid bodies, which arrange themselves around the central mass of pigment, "with the regularity of daisy petals around the central disc" (Golgi).

If we continue the microscopical observation of one of these segmented forms, we often see the small pyriform or oval bodies which are arranged in wreath form take on a more globular appearance, become slightly displaced and pushed away from each other, and then, when the thin involucrum formed by the red corpuscle has disappeared, they lose their original regularity of arrangement, and appear simply as little masses of free, rounded bodies near the residual block of pigment.

At this point the sporulation is broken up, and the individual *gymnospores* invade new red corpuscles, in which they begin their regular life cycle.

Sporulation does not always take place with the same regularity, more especially in regard to the disposition of the pigment, which

may collect in two or more masses, or remain in an irregular fashion between the bodies resulting from fission, instead of being centrally or subcentrally situated in one mass. But these are details of no importance. Of more interest is the fact that sporulation may occur in bodies which have not attained the size of normal adult parasites, but which are decidedly smaller than the corpuscle containing them, of which as much as a third may persist. However, we have seen that in the other species of parasites, the size of the adult bodies in segmentation may vary between wide limits—indeed the quartan parasites show more regularity than do the others in this matter.

The *structure of the quartan parasites*, as seen by Romanowsky's method, is identical with that of the other species. The young and the developing forms have a pigmented cytoplasm and a nuclear formation consisting of a little body of chromatin surrounded by a pallid zone. The line of demarcation between the clear zone (nuclear juice) and the cytoplasm is very distinct. In an advanced stage of development the chromatin, instead of being gathered into one deeply stained body, is arranged in rods and filaments. The segmentation of the nuclear chromatin, by which the nucleus divides into two, four, etc., occurs in the manner described in regard to tertian parasites. In bodies in which we witness the splitting up of a mass of chromatin into two parts, we see the mass assuming irregular and dentilated outlines, showing probably that it is made up of small filaments of chromatin. Around the individual masses of chromatin resulting from the successive divisions, the pallid zone is always visible, although it is thinner and has less distinct outlines than in the young forms. The individual gymnospores are composed of a cytoplasm which stains a deep blue, and of a small excentrically situated body of chromatin of compact appearance (without recognizable structure), surrounded by an extremely thin light zone. This chromatin body corresponds to the shining spot seen in the spore in fresh preparations.

In all essential points this structure, as shown by Romanowsky's method, corresponds to the description of quartan parasites, as seen by the method of Grassi and Feletti. According to these writers the young quartan parasites which have just entered into a red corpuscle consist of a relatively large, excentric nucleus, surrounded by a scanty cytoplasm, and furnished with a delicate membrane, containing the nuclear juice and the so-called *nucleoliform node*, which represents the chromatin substance of the nucleus and lies close to the nuclear membrane. The cytoplasm may possess an alveolar structure. With the development of the parasite the cytoplasm grows more than

does the nucleus, and when the hæmamœba has reached a certain size, we often note the appearance of filaments which unite the nucleoliform node to the nuclear membrane; the node and the filaments represent the so-called nuclear reticulum. Later the nucleoliform node increases in size, and then divides into four, five, eight, or ten little nodes, each one of which becomes surrounded by nuclear juice and a very delicate membrane. What becomes of the reticulum and nuclear membrane during multiplication the authors have not been able to ascertain. The amœba thus becomes multinuclear. Later, a little cytoplasm forms around each nucleus, and thus is formed the complete gymnospore. This process seems to indicate to these authors that the hæmamœba is reproduced by *direct division* of the nucleus.

By Romanowsky's method we do not see the nuclear membrane, whose presence can at the most be merely deduced from the distinct line of separation of the clear zone from the cytoplasm, nor do we perceive the nuclear reticulum from the nucleoliform node, etc. Moreover by the use of Romanowsky's method, it would appear (as he himself believes) that multiplication occurs, not by direct division of the nucleus, but by a rudimentary form of karyokinesis.

Forms which Begin in Man the Cycle Completed in the Mosquito.

These are but little known, as is also the case with the cycle of the quartan parasite in the mosquito.

In preparations made by Romanowsky's method, we see adult forms which take up nearly the whole of the substance of the red corpuscle, which have irregularly disseminated pigment and abundant nuclear chromatin arranged in threads or rods. As in these forms, no matter how large they grow, we see no sign of division of the nucleus, while in bodies of equal size which end in sporulation the nuclear division is already well advanced, it is natural to suppose that they remain sterile in man, and are analogous to the large pigmented tertian forms which are also sterile in man.

In fresh preparations we may see these adult bodies undergoing degenerative processes similar to those which we have described for the tertian.

That the quartan parasites may give rise to *flagellated forms* is a well-established fact; yet, according to our experience, it is rare to find this phase of life. While the patient investigation of a case of estival or of tertian infection is sure to reward us, at a given period of the disease, with a view of the forms known as gametes, and of the flagellates especially, we may follow the course of a quar-

tan for weeks without finding even one. Bastianelli and Bignami were perhaps the first to speak of flagellate quartan bodies. Thayer and Hewetson, who found them in two out of five cases, describe them as smaller than those of the tertian, and as differing in the nature of the pigment which is found in the body from which arise the flagella, the pigment granules being larger and blacker. The movements of the flagella are apparently not slower than in the tertian. On the whole, according to these authors, the quartan flagellated body in its size and in the nature of its pigment, resembles those of estival rather than those of tertian origin.

As to the biological significance of these bodies, our opinion that it is the same as that of similar bodies of estival and tertian parasites is based upon analogy.

Life Cycle of the Malarial Parasite in Mosquitos.

History.

There have been many researches in regard to the malarial parasite outside of the human organism, and these researches have been pursued in various ways. Thus while some have attempted to reach a knowledge of the extracorporeal forms of these parasites by starting from the known forms in man, others have sought for them directly in the air, the dew, or the water of marshes. The object of the first was to cultivate the human parasites in various culture media, modifying in various ways the ordinary media used in the study of bacteria. This was the method used by Marchiafava and Celli in their earliest experiments. The second class of investigators searched in the atmosphere and in marsh water for free living organisms resembling the parasite in man; this was done by Laveran, who speaks of finding motile filaments in water, similar to those in malarial blood. Others, as Silvestrini, injected the washings from malarial earth, marsh water, etc., under the skin in order to ascertain whether they could in this manner produce a malarial disease. Similar experiments were made upon birds by Celli and Sanfelice.

The absolutely negative results obtained by these attempts, including those of Grassi and Calandruccio, who for a while thought that they had found the malarial parasite existing freely in the earth, caused several investigators to think that their researches must be turned in other directions in order to solve the problem, and that possibly the plasmodia were not to be found in a free state outside of man, but as parasites in other animals.

The suspicion that there is an intimate connection between malaria and certain insects is not a new one, and indeed in certain localities it is the popularly held opinion. In these days, since the theory has been proved to be a fact, various authors, as is usual in such cases, have endeavored to find in earlier writers the first allusions to the victorious hypothesis. Thus, for instance, Nuttall quotes from an American author, King, who in a work published in 1883, previously unknown to European readers, clearly expresses the mosquito theory. A patient investigator could, however, easily go much farther back and find this hypothesis.

Without entering into what has been written by certain Italian authors, as Varrone, Vitruvio, and Columella, upon the probable relation between malaria and certain insects, we must not omit to mention what was thought about mosquitos by the celebrated Roman physician, Lancisi, at the beginning of the eighteenth century.

Lancisi, who actively upheld the hypothesis of a close connection between malarial fevers and marshes, attributed to the effluvia of the latter their injurious effects upon man. From a perusal of his work we gather that he attached importance to everything which, originating from stagnant waters, can in any way attack man, including insects and especially mosquitos, to which he pays particular attention, noting their abundance in marshy regions, and above all the abundance of the "vermiculi," whose transformation he noticed "in stridulos culices."

He queries as to the possible manner in which these insects may have an injurious influence on the inhabitants of malarial localities, and assumes that there may be several ways. He expresses the suspicion that the injurious action may be due to the ingestion of waters rendered foul by the insects; but (which is of more interest) he also admits that they may do harm by their sting—not simply by stinging, but by the injection of a toxic substance in the act of puncturing (*non vulnere, sed infuso per vulnus venetico liquido*). Elsewhere he adds that the insects—the "animated effluvia" of marshes—may vitiate our systems not only "per se ipsa irritanto," but also, which is worse, "pravos suos succos cum nostris liquidis permiscendo." In these words and in other parts of Lancisi's work, we find the germ of many recent theories and discussions. Side by side, to be sure, with phrases and sentences in which we seem to recognize a happy intuition of the truth now demonstrated after the lapse of many years, we find contradictory assertions which lack any basis of fact, so that it is difficult to form an exact idea of the author's opinion. This would require a more minute analysis of his work than is possible in this connection.

Up to the present time the majority of observers maintained, as did Lancisi, a cautious attitude, admitting that the vehicles of infection might be many, and that the parasites might be found in both the earth and water of marshy localities, and might be communicated to man in breathing and through the ingestion of infected water. This was the attitude of Laveran, who with Lancisi held that man can be infected in various ways, and he searched for the parasites in water, which he considered to be the chief, if not the only vehicle of infection; as to mosquitos, he emitted the hypothesis that they may take the parasite from man and then infect the water, as they do in the case of filaria, but he did not even hint at the possibility of inoculation as did Lancisi in the previous century.

It is only in the most recent years that the mosquito theory can be said to have really entered the realm of scientific discussion, since no hypothesis can be properly called scientific unless it is sustained by a sufficient number of arguments to give it some probability, taking into consideration the condition of knowledge at the time of its inception and when demonstrative facts are sought.

At the present time there are two methods of research: one followed by Bignami, who, endeavoring to ascertain how fevers are taken, showed how great are the difficulties met with in considering the air and water as vehicles, and demonstrated the probability of inoculation by the mosquito, dwelling upon the analogy of human malaria with Texas fever; moreover, since 1894 he has endeavored to prove these views by experimental demonstration. The other method has been followed by Manson, who, taking up Laveran's theory, tried to find out what were the forms of human parasites which were capable of passing into mosquitos and there continuing their development. He considered the flagella to be spores, which becoming freed in the mosquito's intestine from the cysts containing them, continued to develop, and at the death of the mosquito became free in the water, which thus was converted into a vehicle of infection to man. These two theories seemed at first to be so absolutely contradictory that they gave rise to written discussions which have certainly been of use in stimulating the study of the question. Especial subjects of discussion were the significance attributed by Manson to the flagella, which is not upheld by fact, and the importance attributed to the Laveran-Manson theory of water as a vehicle of infection, which is contradicted by accurate epidemiological observations, and by experimentation in the School of Hygiene of Rome. But further research has led rapidly to harmony in fundamental opinions, by demonstrating that *mosquitos take the parasite from man and inoculate man with it again.*

Manson's theory had the great merit of serving as guide to the researches of Ross, who by causing birds infected with *proteosoma* (Labbé) to be stung by a species of mosquito (gray mosquito), determined in the latter the forms of a new parasitic life cycle. These were found in the walls of the middle intestine where, according to Ross, the proteosoma assumes the aspect of a coccidium (proteosoma coccidia); in the mature capsules of these coccidia were formed germinal corpuscles (*germinal rods*), which accumulated in the poison-salivary glands of the gray mosquito, which at this point became capable of infecting healthy sparrows with the proteosoma (1898).

By these researches, which gave us our first information upon the life forms of a hæmosporidium in the body of a mosquito, was demonstrated the important fact, that not every species of mosquito can give lodgment to a given hæmatozoon; in fact, Ross found the developmental stages of his proteosoma coccidia only in the gray mosquito.

This rendered it more than probable that only a determined species of mosquito can transport the infection to man; whence the necessity of a preliminary zoological study upon mosquitos of malarial regions, with the view of ascertaining the dominant species. With this idea Grassi, investigating the distribution of mosquitos in malarial regions in the summer of 1898, came to the conclusion that in malarial countries, in addition to the species found in non-malarial regions, there are others which are completely absent from the latter places. In non-malarial countries the *Culex pipiens* and other species of Culex predominate; in malarial regions we find in large number the *Anopheles claviger*, other species of Anopheles, the *Culex penicellaris*, and other species of Culex which, the mosquito theory of the origin of human malaria being once admitted, must naturally be open to suspicion.

It was therefore to be expected that the attention of observers should be attracted to these species, and that they should be made the subject of further experimental researches. With these Bignami obtained in Rome the first case of experimental malaria in man; this was reported in November, 1898. In rapid succession there followed the observations of Grassi and of Bignami and Bastianelli upon the development of human parasites in mosquitos of the genus *Anopheles*, and especially in *A. claviger*, which was the chief one to attract the attention of these observers, principally because of its abundance in the Roman Campagna.

From the various reports upon this subject published during the past few months by Grassi, and Bignami and Bastianelli, who have demonstrated that the parasites of human malaria pursue in the Ano-

pheles a life cycle similar to that described by Ross in the case of the proteosoma of birds, we take the facts described in the following pages.

The Malarial Mosquitos.

From what has been said, it is evident that only certain determined species of mosquito can harbor the malarial parasite of man. From researches recently carried on in Rome we see that in all the species of the genus *Anopheles* we are able to follow the whole cycle of development of the parasite; in no species of the genus *Culex* have any forms of development of the hæmosporidia been found. It is to be noted, however, that researches with the last-named species have not been many, and that they are still under way, so that we cannot positively exclude the possibility of such development.

We will briefly describe the characteristics of the malarial genus and species, taking our description chiefly from Ficalbi's work. For further details in the description of each species we refer the reader to that work.

Genus Anopheles, Meigen (1818).—From the Greek ἀνωφελής, injurious. *Palpi in both sexes, about as long as the proboscis.* The palpi in the female are four-jointed, but in the basal joint there is a constriction towards the root which apparently forms a basal articulation, and gives the palpus the appearance of being five-jointed; another constriction sometimes makes it seem six-jointed. The palpi of the male are really three-jointed, but appear four-jointed by reason of a constriction in the basal portion towards the root; and sometimes the presence of two constrictions, one towards the middle of the long portion and one in the apparent basal joint gives the appearance of five or six articulations. The appearance of five joints in the female and four in the male is the usual one. In the female the palpi resemble straight filaments, which in repose are parallel with the proboscis, forming with it a bundle of three parts; when the female stings, they rise and diverge; in the female the antepenultimate joint is as long as, or longer than, both the penultimate and ultimate. In the male the palpi in the last two joints are short, thick, and olive-shaped. The nucha has a posterior crown of scales. The abdomen is pilose on both its dorsal and ventral surfaces, but there are no squamæ, which are abundant in the genus Culex. The legs are very long, ending in ungues or in simple or dentated claws.

There are in Europe five species of Anopheles, which are divided into two groups according as to whether the wings are spotted or not.

I. Wings Without Spots.—*1. Anopheles bifurcatus*, Lin. (1758). Wings without spots. Species less black than the following, of me-

dium size (7.5 to 10 mm.). Some individuals may be smaller and brownish-black, some larger and brownish-yellow. A somewhat diffuse species, from Northern Europe to Southern Italy and the Italian islands, including Sardinia. In the Roman Campagna it is found in much less abundance than A. claviger.

2. *Anopheles villosus*, Robineau Desvoidy (1827). Resembles the bifurcatus, but is larger and more pilose. Ficalbi thinks that possibly it may not be a species, but a variety of A. bifurcatus.

3. *Anopheles nigripes*, Staeger (1839). Wings without spots, like the preceding. Proboscis, palpi, antennæ, tibiæ, and tarsi blacker than in A. bifurcatus. Size smaller (not more than 8 mm.). According to Ficalbi, it belongs especially to Northern Europe, and is rare. Ficalbi thinks that instead of constituting a distinct species it may be a small and dark specimen of bifurcatus which he has had frequent opportunity to study.

These three species (or varieties ?) differ, therefore, as we see in very small and unimportant details. We note that A. bifurcatus, upon which experiments have been made in Rome (it is caught in the Roman Campagna or in the neighborhood), resembles more the variety nigripes.

II. Spotted Wings.—4. *Anopheles claviger*, Fabricius (1805); *A. maculipennis*, Meigen (1818). Wings with four spots formed by masses of chitinous squamæ. Femora of the anterior pair not enlarged at the base. The description of A. claviger agrees with that of A. bifurcatus with the exception that A. claviger has the spotted wings and has a rather more yellowish appearance.

For a more detailed description we refer, as throughout, to Ficalbi. We would only remark that the wings are brown, especially in the female, or slightly yellowish-brown; even with the naked eye we can distinctly see the four black spots, which are as a rule more conspicuous and better developed in the female than in the male, and are so placed that if joined by an imaginary line they would form a capital L. Under the magnifying glass we see that the wings are rich in black scales, an accumulation of which produces the spots. Total length of body, including proboscis, of the female is 7.5 to 9 mm.; the male is always smaller than the female.

This species is diffused throughout Europe, having been found in Scandinavia, England, Austria, Germany, Russia, etc. Ficalbi has found it largely disseminated in Italy, where it is the most common species of the genus Anopheles, and is commonly called *zanzarone* (big mosquito). It is especially to be noted that *this species is found in great numbers in well-watered plains.*

5. *Anopheles pictus*, Loew (1845). Wings, even to the naked eye,

seem to have blackish-brown and rather tawny lightish-yellow spots, due to accumulations of squamæ of these colors. Femora of the anterior pair slightly enlarged in the proximal third.

Omitting a detailed description we would note that the anterior margins of the wings as far as their tips are of a blackish-brown color, which is interrupted by three yellowish marginal spots. Of these three colored spots the centre one is the largest, the posterior

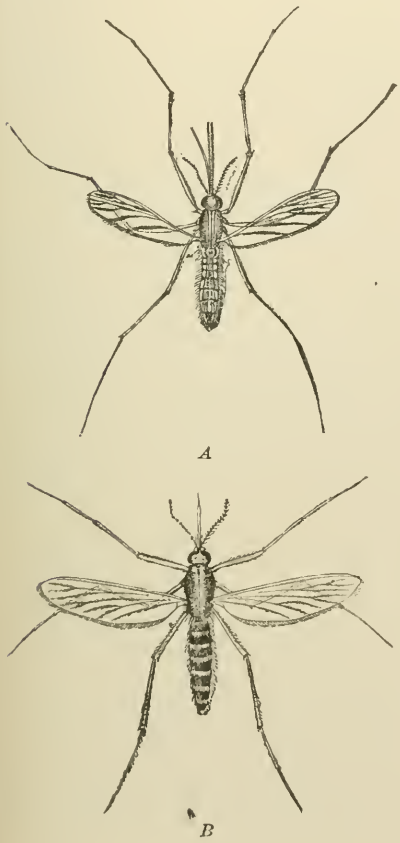


FIG. 1.—Mosquitos. *A*, Anopheles claviger; [*B*, a specimen of culex caught in the neighborhood of New York; *C* shows the different positions assumed by Anopheles and Culex when at rest on a wall; according to Strachan and others (*British Medical Journal*, September 30th, 1899; *Journal of Tropical Medicine*, December, 1899), in the attitude of rest the axis of the body of Anopheles is almost vertical to the wall, while that of Culex is parallel to the surface on which the insect rests].

the smallest, the latter not touching the alar margin. In the remaining portion of the wings the black squamæ of the nervation accumulating at certain points, and alternating with yellow squamæ, produce five, six, or seven brownish spots.

The posterior margin of the wings has black scales, which at a point corresponding to the anterior third of the margin are of a tawny lightish-yellow color, forming marginal spots of this color.

The total length of the female, including the proboscis, is from 7 to 8 mm. (Ficalbi).

Loew caught only males of this species on the coast of Asia Minor, opposite the island of Rhodes, and thought it indigenous to Southern Europe. Later Ficalbi captured only females of the species in Tuscany in the forest of Tombolo near Pisa, in the summer.

The mosquitos with spotted wings upon which Ross experimented in India, as seen by the study of a specimen sent by Ross to Grassi in Rome, belong to the genus Anopheles, and are very similar to, if not identical with, *A. pictus*. Ross distinguishes two varieties—*small* and *large dappled-winged mosquitos*. In Italy also there are small and large ones that Grassi regards as different species of Anopheles, distinguishing them not only by their dimensions, but also by the designs on wings and palpi. The *A. pictus*, spoken of above, corresponds to the small form. The large variety in Italy, called by Grassi *Anopheles pseudopictus*, has a total length of about 11 mm.

As to the *life* and *habits* of this species of mosquito, we possess little knowledge. The facts observed during the past year in the Roman Campagna refer almost exclusively to *Anopheles claviger*, to which (according to the most recent researches) belongs the chief rôle in the transmission of malaria, at least in these regions. In fact, the development of all three varieties of the malarial parasite of man has been observed in this species of mosquito. It is moreover the most common and the most numerous of the species of the genus Anopheles in localities of grave malaria in the Campagna, as Ostia, Porto presso Fiumicino, Maccarese, etc.

In the spring, summer, and autumn we find in the fields many kinds of mosquito belonging to the genus Culex, in addition to the Anopheles. But as the season advances, especially when the temperature begins to be lowered, the *A. claviger* begins to predominate in the houses, huts, and stables in the country. In late autumn and winter people sent out to collect mosquitos capture almost exclusively the fecundated females of *A. claviger*, if they limit their search to dwelling-houses and stables. This is because with the first advent of cold weather the fecundated females prepare to hibernate, as nearly all mosquitos do, and take refuge in enclosed places where there are animals and men; where the winter season is mild, as on the coast of Southern Italy, we find females of *A. claviger* hibernating in caves. The males disappear, and it is evidently only the fertile female that hibernates. These hibernating females, when gathered up and taken to laboratories with a temperature of about 20° C. (68° F.) or even lower, awake, become very lively, and sting as usual.

The species of the genus Culex (as *C. pipiens*) appear to hiber-

nate by preference in grottoes, in the trunks of trees, under bushes, etc. The females of *A. pictus* do not hibernate in houses; during the past winter they were found in caves in Southern Italy.

With the first warmth of spring the females fly about and sting as usual, and then lay their eggs. Females transported to the laboratory and kept at a temperature of between 20° and 22° C. (68° to about 71° F.) laid eggs, as witnessed by Bastianelli and Bignami, in the month of March; the larvæ were seen a few days later, and developed in from fifteen to twenty days. The insect remained in the chrysalis stage for four to five days; thus after about twenty-five to thirty days in all, the insect emerged from the puparium. Four or five days after birth the females were capable of biting and of extracting blood.

The eggs are easily distinguished from those of *Culex pipiens;* they are shaped like an elongated spindle with two lateral wings, and are deposited in strings; in each string the individual eggs are placed transversely, and touch each other in the direction of the long axis; they do not therefore have the heaped-up appearance of those of *C. pipiens.* The larvæ are also easily distinguished from those of *C. pipiens.* Omitting the zoological characters, into a description of which we have not space to enter, we would merely state that they are brown, very agile, and always move in a horizontal direction, never in a vertical or oblique one, as do those of *C. pipiens.* They live in stagnant, preferably in deep, water, and if the surface of the water be even slightly agitated they take refuge at the bottom. They are usually found isolated or in small groups, not gathered into large masses like the larvæ of *C. pipiens.* Ficalbi says that they do not choose such dirty water as those of the genus Culex frequently do, but often live in rather clear water. We have, however, seen them existing and developing perfectly in very dirty stagnant waters, swarming with every kind of insect life.

The males are herbivorous. The females alone suck blood, and they are most voracious. For example, while we can rarely make an individual of the genus Culex bite by enclosing it in a glass tube the open end of which is in contact with the skin of a human being, the female of *A. claviger* is easily induced to bite under these conditions. They also attack domestic animals, preferably the horse. We have found them in great abundance in stables, at Porto for instance.

In some persons their bite produces a persistent wheal which causes the most troublesome itching; in many persons, however, the bite leaves no trace at all, and there are even individuals (altogether exceptional) who for some unknown reason seem never to be bitten.

As to their habits, they are, so to speak, country mosquitos, and

prefer localities rich in water; they stop, in fact, just at the threshold of houses, and in all our researches made in Rome during the past year we have never found non-hibernating mosquitos of this species within the houses adjoining gardens in which they were to be seen in abundance. They are found, however, in localities at a little distance from the city, as, for instance, in a stable near the Porta del Popolo.

We do not know by direct observation whether they are able to migrate, and if so, whether they go far, or what conditions govern such migration. We do know, however, that they can be transported from unhealthy to healthy localities, as, for instance, in hay from malarial fields into the city. This circumstance will serve to explain the isolated cases of malaria which are sometimes seen to occur in healthy localities.

We have said that the fecundated females after hibernation seek water and there deposit their eggs, so that in the spring we already have the new generation of winged insects. These during the hot season may give rise to several generations. Mainert found semi-adult larvæ as early as the middle of March in warm years; in July and a little later in the summer a second generation of adult larvæ was found; and in one year in which the spring was very early, he found at the end of October small larvæ belonging to the third generation. It is probable that this third generation lives out its full cycle only in southern countries.

Methods of Research.

The development of the malarial parasites of the mosquito has been systematically studied by the authors above quoted, by causing adult patients to be bitten by mosquitos enclosed in glass tubes, the mouths of which were applied to the skin until the enclosed insects had satisfied their appetite. The mosquitos were then set free either under a netting or in a large glass jar, in which were placed blades of fresh grass and a few drops of water at the bottom. The atmosphere was kept at a constant temperature of about 20° or 22° to 30° C. (68° to 86° F.).

As the life phases of the parasite are observed in the mid-intestine and in the salivary glands, the preparation of these parts is of great importance.

To prepare the middle intestine, the mosquito is anæsthetized by ether or tobacco smoke, and then fixed upon a piece of colored glass by means of a needle passed through the thorax, with the back towards the glass, then with teazing needle we press lightly at about

the third abdominal segment, and very gently push apart the two needles, making slight traction. By this means we draw out the whole intestine; the anterior intestine is ruptured at the thoracic segment, the posterior intestine remains adherent to the last abdominal segments, which are detached from the others, and the middle intestine—the most important for our researches—remains free. All this takes place in a small drop of physiological salt solution or in a weak solution of formalin (one to two per cent.).

To prepare the salivary glands we proceed as follows: Holding the thorax fixed, as described, we try to detach the head by slight traction with the needle, and thus sometimes succeed in extracting all the glandular tubes with their excretory ducts; if this does not occur, we shall have to tear off the anterior half of the thoracic segment with two fine teazing needles.

Fresh preparations, obtained in this manner, are examined in the sodium-chloride or the formalin solution.

To obtain stable stained preparations, we leave the same organs adhering to the glass, and fix them with a five-per-cent. solution of formalin or a saturated solution of bichloride of mercury; then the specimen is passed through alcohol, and stained by Böhmer's hæmatoxylin or by the ferric hæmatoxylin of Heidenhain. To obtain clear preparations of the parasite in the middle intestine, it is well to detach the epithelium before fixation, for as these cells take on a deep stain they naturally interfere with the examination.

Another method is to fix the whole insect in bichloride solution, enclose it in paraffin, make sections of it *in toto*, and stain the sections in one of the above-mentioned ways. From preparations thus obtained we get a clear idea of the relations of the parasite to the intestine of the mosquito and to the surrounding parts.

For a recognition of the parasite the magnification obtained by the ordinary dry lenses is sufficient; to study its structure in stained preparations we need a homogeneous immersion lens.

Cycle of Development of Crescent Forms in the body of Anopheles Claviger.

The ulterior development of the crescents has been followed by Grassi, and Bignami and Bastianelli, in *A. claviger*, *A. bifurcatus*, and *A. pictus*. We would add that Ross has seen the first stage of development of the crescents in a form of *dappled-winged mosquito*, which was recognized by Grassi (in a specimen sent by Ross to Rome) as much like *A. pictus*. In Rome it is only in *A. claviger* that the complete cycle of development of the parasite has been fol-

lowed. The structure of the parasite in its various evolutionary phases has been studied in stained preparations, and it has been recognized as possessing the characters of a typical sporozoon.

We have already described the modifications undergone by crescent forms in blood taken from man, the formation of the pseudo-flagellate bodies, etc., giving the reasons and the data which lead us to consider these forms as *gametes*. We also stated that the natural medium in which is completed the formation of the *microgametes* (flagella) and, as we have every reason to think, fecundation occurs, is the mid-intestine of some species of mosquito. By direct research it has been in fact established that in the blood contained in the mid-intestine of Anopheles some crescents become flagellated, and even individual flagella have been seen. Up to the present time, however, it has not been possible to witness the phenomena of fecundation, nor to see the nuclear modifications that, as we are led to infer from the analogy of similar processes in other sporozoa, must take place in the fertilized crescents.

Not in every case in which a patient with crescent forms in the blood is stung by Anopheles do we obtain their regular development, nor do all the crescents develop in every case. It is in fact necessary that the crescents should be in the adult stage, and we might say *mature;* only when in the examination of the blood we find forms of the crescent phase that rapidly become flagellated do we obtain their regular development in their new host.

The following description relates to the development of crescents in *A. claviger* kept by the thermostat at a constant temperature of 30° C. (86° F.). If we examine the middle intestine of an individual of *A. claviger* a little less than *two days after* it has sucked in crescent blood, we find in its walls fusiform bodies which in a fresh preparation appear to be identical in form and appearance with the spindle-shaped bodies found in human blood; they differ from them only in being a little larger, and in having a different arrangement of the pigment. In preparations stained with hæmatoxylin these bodies exhibit a large nucleus with a mass of central chromatin which may be round or elongated; the protoplasm appears to be vacuolated. More rarely we see pigmented bodies possessing the same characters, but ovoid or roundish in shape. The pigment, in both spindle and ovoid bodies, is found to be identical with that of the crescent forms, and for the most part is situated at the periphery of the parasitic bodies.

The development of these bodies is best studied by examination of sections *in toto* of the mosquito, and we find its seat to be on the outside of the epithelium and basement membrane between the

cells of the adipose tissue and the muscular fibres of the intestinal walls.

On the *third* and *fourth* days the parasites are found to have markedly increased in size, and to possess a protoplasm with a reticular aspect, the pigment being apparently in smaller amount and irregularly disseminated. The parasitic body is clearly seen to be enclosed in a capsule.

On the *fifth* or *sixth* day the parasites have enormously increased in size, up to 70 μ or more, and project from the walls of the intestine into the cœloma, and may easily be seen even under a low power. In their interior may be seen numerous small bodies which in stained preparations are recognized as nuclei, and shining bodies resembling fat which in part existed in the previous phase. The capsule is more visible.

At about the *seventh* day the parasite contains an enormous number of delicate, thread-like filaments with thinned extremities, about 14 mm. in length, arranged like rays around one or more homogeneous masses in which a little black pigment is still to be seen. If they are crushed in such a way as to break the capsule innumerable filaments which are all alike make their exit. In the centre of these, in preparations stained with hæmatoxylin or after Romanowsky's method, will be seen one or more granules or small bodies of nuclear chromatin.

The individual forms so far described evidently represent the developing stages of a sporozoon, which go on to maturation. This development consists essentially in an increase in size with encapsulation, and in successive multiplications of the nucleus, up to the formation of very small nuclei, around each of which is gathered a little protoplasm (sporoblast without capsule). The sporoblasts are transformed into the filiform elements described—the *sporozoites;* so that the mature sporozoon (seventh day) is composed of a thin capsule with innumerable sporozoites and the residue of segmentation (*nucleus de reliquat*).

In the succeeding days we find the torn and flaccid capsular membrane adhering to the intestine, and near to it the sporozoites which later accumulate in great number in the tubules of the salivary gland, or within the cells of this gland, or in the glandular lumen. At this point the Anopheles, biting a healthy man, inoculate him with the sporozoites along with the saliva, thus determining an estivo-autumnal fever, after a period of incubation. This has been experimentally demonstrated, as we shall see in the following section describing inoculation experiments.

Such is the life cycle of the crescent forms in the body of *A.*

claviger. It is exactly similar to that observed by Ross in regard to the proteosoma of birds in the *gray mosquito*.

As to the time necessary for the completion of the cycle, we must note that the temperature exerts a certain influence. At a temperature of 20° to 22° C. (68° to 71.6° F.) the development is much slower than that which we have described; at 14° to 15° C. (57° to 59° F.) it would appear from the observations so far made that the development does not occur at all.

In addition to the forms described, we may find within the capsule, in the mid-intestine, peculiar brown bodies, varying in size and irregular in shape, some like rods, others ovoid or round, some straight and others curved. These bodies, like those seen by Ross in studying *Proteosoma coccidia*, may be found within broken or shrunken capsules or else within a large capsule which is apparently distended by its contents. The irregularity of these bodies and their sometimes stratified appearance lead one to think that they are the product of retrogressive changes in the sporozoa.

Development of Tertian Parasites in Anopheles Claviger.

The development of the large pigmented bodies of tertian infection has been seen, so far, by Grassi, and Bignami and Bastianelli in *Anopheles claviger* and *A. bifurcatus*.

Bastianelli and Bignami have followed the whole life cycle of the parasite in *A. claviger*, wherefore we will take the following description of it from their work.

We have already studied the formation of the macro- and the microgametes, and described their structure, adding that the ulterior life phases of these bodies are normally developed in the mid-intestine of the Anopheles. Here occurs the fertilization of a macrogamete by a flagellum (microgamete), and the fecundated body then penetrates into the intestinal wall where it continues its development. In truth, up to the present time no one has had the opportunity of actually witnessing the process of fecundation, nor of following the first succeeding nuclear changes; therefore our theory that the sporozoon developed in the Anopheles is the fecundated macrogamete is founded upon analogy, as we have already stated.

In the Anopheles which have bitten a tertian patient having in his blood the forms regarded as gametes, and which are kept for *about forty hours after the puncture* in a constant temperature of about 30° C. (86° F.), we can easily see a certain number of tertian bodies in the thickness of the walls of the middle intestine, especially in its ter-

minal portion. They appear as round, pigmented bodies, very transparent and with distinct outlines, and contents varying in appearance, sometimes uniform, sometimes vacuolated, or else divided into masses. They are easily recognized by the characteristics of the pigment, which are those typical of tertian pigment, which is usually immotile and only exceptionally in motion. In preparations stained with hæmatoxylin we see that the protoplasm has a reticular aspect, and that the chromatin has increased in amount (relatively to the amount seen in the same bodies before they have penetrated the intestinal wall), and not rarely it is undergoing division or has divided into various little masses. At this stage of development the diameter of the parasite is one and one-half to two times that of a red blood cell.

On the *third day* after the puncture the parasites are from one-fourth to one-third larger than the preceding day. They possess a very evident cystic wall, and in fresh preparations their contents are usually seen to be divided into little masses, between which is the pigment. In stained preparations we find a varying number of round, ovoid, and deeply colored nuclei (from eight to fifteen).

On the *fourth day* the size of the cystic body has increased about one-fourth, and the cyst wall is very distinct. The nuclei are more numerous (twenty to thirty) and somewhat smaller than in the preceding stage. The protoplasm preserves a reticular, almost spongy appearance.

Between the fourth and the fifth days the parasites which from the beginning have been situated outside of the intestinal mucous membrane, between the muscular fibres of the walls, begin to project between the fat cells into the cœloma. They continue to increase in size, so that they are easily visible with a low-power dry lens. In fresh preparations they look like the bodies of the preceding stage, and in stained preparations we find that the nuclear division continues, so that there is a constant production of more numerous and smaller nuclei.

As a rule *on the third day* we already see within the capsule distinct groups of filaments (sporozoites) arranged side by side like a palisade, and in groups around masses of an apparently amorphous substance. At this time some capsules may in fresh specimens appear to be almost entirely filled with sporozoites; but in stained preparations we recognize the presence of amorphous masses which are usually multiple, and which represent the residua of segmentation.

The structure of the sporozoites is like that already described for the mature sporozoa of crescent origin. We must observe that the

description given above and taken from the work referred to (in which the reader will find further details) is somewhat schematic as regards the size of the body and the stage of development in the several days after the puncture. We may, in fact, observe cystic bodies about as large as those of the fourth day, already mature, that is to say, filled with completely developed sporozoites. Moreover, we often see in mosquitos which have bitten a patient once only, cystic bodies in various stages of development and of different sizes.

It is therefore evident that the development of the malarial parasites does not occur with the same regularity as to time in the intestines of the Anopheles as it does in the blood of man.

After about the seventh day we find the broken and shrunken capsules in the intestines and the sporozoites in the cells or the excretory ducts of the salivary glands. In the latter the sporozoites are either of the form that they are in the capsule in the intestines or else shorter and thicker.

In mosquitos nourished with tertian blood, there have not been found the *brown bodies* described by Ross in the so-called *proteosoma coccidia,* and found in Rome in the crescent sporozoites.

Difference Between the Crescent and the Tertian Sporozoa in the Anophelic Life Cycle.

There are certain differences here which, although slight, admit of a differential diagnosis in some stages of development at least. According to Bastianelli and Bignami these are as follows:

(*a*) *Form of the Sporozoon in the First Stages of Development.*—In the crescent this is either spindle or ovoid, while in the tertian it is round, or, in exceptional cases, oval. In the rare cases in which it is oval, this may possibly be due to stretching during extraction from the intestines.

(*b*) *Appearance of the Sporozoon.*—The crescent sporozoon at the same stage of development has a more distinct outline and greater refractive power, so that it is well seen even under a low power, while the tertian is more transparent and in the first stages is visible only by a high power (homogeneous immersion).

(*c*) *Quality of the Pigment.*—This is naturally identical with that of the corresponding parasites in man.

(*d*) *Size and Number of the Nuclei Produced by Successive Divisions.*—The nuclei of the tertian sporozoon are less numerous and larger than those of the crescent sporozoon at the same stage of development.

(*e*) *Disposition of the Sporozoites within the Capsule.*—In the ter-

tian sporozoon the sporozoites are less dense and more regularly arranged, sometimes in rays around the residua of segmentation, than in the capsule of crescent origin. The residua of segmentation in the case of tertian parasites are usually composed of several granular blocks which are more numerous than in the case of crescent parasites. There are, however, mature capsules which exhibit no appreciable differences.

Development of the Quartan Parasite in Anopheles Claviger.

Although the authors quoted above made rather numerous experiments with the quartan parasite, they succeeded only once in obtaining a positive result. In one *A. claviger* nourished upon a woman who had suffered eighteen months from quartan fever, and who had many parasites in the blood with a few rare gametes, there were found two capsules containing the characteristic pigment of the quartan parasite. These capsules when three days old had about the same dimensions as the two-day capsules of crescent origin. The negative results obtained in the other researches are probably due to the extreme rarity with which flagellated bodies are found in quartan blood. Possibly in this species of infection in which the parasites grow so flourishingly in human blood, there may be so complete an adaptation of the parasite to this mode of existence that after a while it may entirely lose the power of producing bodies capable of ulterior development in a different atmosphere. But this is a theory upon which we do not insist, but it may be proved or disproved by facts before long.*

The Possibility of Other Life Cycles.

Do the forms which we have described for the crescent and tertian parasites represent the only life cycle of these beings, or are there forms belonging to another cycle?

The above-mentioned authors, in their fourth preliminary note relating to the development of the crescents in *A. claviger*, described, in addition to the forms of which we have spoken, tubular or ampulla-like masses of small round or ovoid bodies, some hyaline, others covered with a dark yellowish-brown membrane; in the same

* From the latest researches made by Bignami and Bastianelli, who have succeeded in observing the whole life cycle of the quartan parasite in A. claviger, it appears that positive results are obtained only in cases which have lasted a very long time. When recent cases are employed for experiment the results are negative. Also, the gametes would seem to make their appearance only in cases which have had several relapses.

preparation, indeed, we may see the various phases of development of thick membrane which surrounds the hyaline body. These bodies, which are found within the intestine or in the dorsal vessel, appear to be resistant spores, and as they greatly resemble the brown bodies of unknown significance which are found within the capsule of malarial sporozoa, the above-named authors at the beginning of their researches held that they were identical with these brown bodies, and considered them to be a resistant form of the malarial parasite in the mosquito—a form capable of passing into water at the insect's death, and then going through with a new life cycle. Later researches, however, having demonstrated that the development of the brown spores from the typical forms of malarial sporozoa cannot be followed, they then held that these were special parasites, probably another parasitic sporozoon of the Anopheles having no relation to the malarial parasite. We must therefore conclude that as yet there is no known form of the malarial sporozoa of the mosquito which has the significance of a resistant spore.

In the second place, can the malarial parasites pass from the infected mosquito to its progeny, through the eggs and larvæ?

This theory having the semblance of probability from what is known of the biology of the parasites in Texas fever, an attempt has been made to solve the question in regard to the parasites of human malaria by two methods of research. On the one hand, the eggs and larvæ of supposedly infected Anopheles were studied, and on the other hand, men were caused to be bitten by mosquitos born in the laboratory, and which were therefore known not to have been nourished by malarial blood, the object being to ascertain whether they bore in themselves germs of infection from their birth.

The result of the first class of researches was that in the well-developed eggs of the Anopheles we not infrequently find cystic bodies containing eight easily colored little bodies, which may be considered as the spores of a sporozoon with eight sporozoites. But it has not been possible to determine whether there is any relation between these bodies and the malarial parasites; in fact, it would seem that there is none. On the other hand, it has not been possible to follow the development of these bodies in the larvæ; neither have there been found in new-born mosquitos any forms at all like malarial parasites.

The second series of researches have also given negative results. Six healthy persons allowed themselves to be bitten many times by Anopheles born in the laboratory, and not one of them had the fever. Researches are still being pursued in regard to this question, which,

as may readily be seen, has the greatest possible importance in reference to the transmission of malarial germs.

We are therefore forced to the conclusion that the only fact determined by the researches so far pursued is that *the malarial parasites pass from the sick person into certain species of mosquitos, and from these, after having pursued the life cycle described, they return again to man.* Whether the information recently acquired in regard to the forms of these two cycles includes fundamentally *all* the biology of the malarial parasite in and outside of man, or whether there are other forms of life as yet unknown, is a question which can be answered only after further investigation.

Systematic Position of the Malarial Parasites.

Malarial parasites have been variously classified by zoologists; the majority place them in the class of the *Sporozoa;* others in that of the *Rhizopoda.*

Grassi, in his earlier researches, classed them with the rhizopoda for various reasons, but chiefly because he thought it certain that these parasites multiplied in the free state, whereas the sporozoa never do. Starting from this conception, he searched for the malarial parasites in the earth and in all the substances which are popularly believed to be malarial.

Among those who have upheld the view that these parasites belong to the sporozoa are Metchnikoff, Danilewsky, and Labbé; and the classification has apparently found a firm support from the recent researches into the life cycle of the parasite outside of man. In mosquitos, indeed, they appear as typical sporozoa.

The class of the Sporozoa (Leuckart, 1879)—protozoa which lead a parasitic existence and multiply by sporulation—has been divided into various orders and sub-classes: *Gregarinidea, Coccidiidea, Myxosporidia, Sarcocystidia, Microsporidia,* to which P. Mingazzini has proposed the addition of the sub-class *Hæmosporidia.* Under the name of *Hæmosporidia* are included all the parasites of the red corpuscles that are found in frogs, reptiles, birds, and mammals.

Some, however, adopt the expression in a more limited sense. Thus Labbé divides the parasites of the blood into two orders, which he classes among the Sporozoa: I. *Hæmosporidia,* and II. *Gymnosporidia.* Among the latter he places the parasites of man, which he calls *Hæmamœba Laverani.*

Other classifications of the blood parasites have been suggested, which we will enumerate in part, leaving it to zoologists to decide upon their merits.

Kruse, Celli, and Sanfelice group all the species under three different genera: 1. *Hæmogregarina* (*Drepanidium*) (parasites of the frog). 2. *Hæmoproteus* (parasites of birds). 3. *Plasmodium* (parasites of man).

Grassi and Feletti divide the malarial parasites and the forms related to them into two genera:

1. Genus *Hæmamœba*, which includes the following species: *H. malariæ* (quartan parasites), *H. vivax* (tertian), *H. præcox* (pernicious), and *H. immaculata* (pernicious). To these may be added another group of species, which are parasites of birds: *H. relicta* (in the sparrow, lark, etc.), *H. subpræcox* (in the lark, owl, etc.), *H. subimmaculata* (in the hawk).

2. Genus *Laverania*, to which belongs a species that lives in man, *L. malariæ* (crescent parasites); and other parasitic species in other animals, *L. Danilewsky* (in many pigeons, sparrows, and birds of prey), *L. ranarum* (in the edible frog).

Laveran is among the few to hold that the parasite of man is a form of a species which is polymorphous—one species with variable development. The various febrile types, he thinks, are not due to differences in the parasites, but to a as yet unknown disposition on the part of the affected organism; in fact, he states that there are many cases of fever in which there is no constant relation between the febrile type and the parasitic form. This last statement of Laveran is contradicted by all modern researches, which will be in part described in the chapters to follow upon the various kinds of fever. To this author's view we may oppose that held in general by the Italian investigators, who, following Golgi, distinguish various species of malarial parasites, in intimate relation with the variety of the fever.

Various classifications of the malarial parasite of man have been proposed. We have already given that of Grassi and Feletti, which cannot be accepted in what relates to man, because they divide the parasites of pernicious fever (*Hæmamœba præcox*) from the crescent parasites (*Laverania malariæ*). This is a view which we have always opposed. It was open to discussion up to a short while ago, but is no longer sustainable since it has been demonstrated that the crescents represent a phase in the life of the estivoautumnal parasites, and in particular that phase which continues its development outside of man and within the mosquito.

Mannaberg divides the parasites into two groups, according to whether or not there is a formation of *syzygies*:

1. Parasites with sporulation without syzygies (that is, without crescent forms): (*a*) Quartan parasites; (*b*) tertian parasites.

2. Parasites with sporulation and with the formation of syzygies

(with crescents): (*a*) Pigmented quotidian parasite; (*b*) non-pigmented quotidian parasite; (*c*) malignant tertian parasite.

As to the fundamental basis of this classification we would observe that it is not possible to contrast the estivoautumnal parasites (Group 2 of Mannaberg) with those of the tertian and quartan by reason of the presence or absence of crescent bodies; indeed it has now been demonstrated that in the tertian, for instance, there are parasitic forms (large mononucleated pigmented bodies) which have the same biological significance and the same ulterior development as the crescent forms. As to the subdivision of the second group, as we have already stated, we hold that there is not as yet sufficient proof to allow of the admission that the parasites which complete their entire cycle without becoming pigmented represent a species by themselves, although the probabilities point that way. In the second place, although we were the first to propose the distinction of the estivoautumnal parasites into *quotidian parasites* and *parasites of the malignant tertian*, we are disposed to look upon these two forms as closely related varieties of the estivoautumnal parasites and not as distinct species.

In the preceding description we distinguished three species of malarial parasite, viz.: 1, estivoautumnal; 2, tertian; and 3, quartan parasites. In this classification we take into consideration only the best proved facts, leaving out all disputed points; so that, if we examine the classifications proposed by the various authors, we find that in spite of divergences they all agree in considering the three species as distinct. We think it probable that the first includes several closely related varieties. But leaving aside these questions of secondary importance, we find that the arguments upon which the division of the malarial parasites into the said species rests are three:

A. The three species exhibit perfect constancy in all essential morphological and biological characteristics, so that they can easily be recognized on microscopical examination, and these fundamental characteristics have been noted in all malarial countries.

B. They are inoculable from man to man, and each reproduces its own form without ever becoming transformed into another.

C. They have an indisputable and close relation with a determined clinical species.

Let us briefly examine into these three points.

A. *The constancy of the morphological and biological characteristics* constitutes a fact which is not only of scientific importance, but is also of great practical value, because it permits us to make with positiveness the differential diagnosis between the three species of parasites described; and this, as we shall see, is of great importance as regards the prognosis.

The quartan parasites differ from the tertian in their morphological and biological properties (Golgi), so that it is always possible and even easy to one expert in examinations to diagnose between the two. There are in fact:

1. *Differences in the developmental cycle.* The ordinary tertian parasite completes its whole life cycle in two days, that of the quartan in three.

2. *Differences in the character of the amœboid movements.* The endoglobular amœboid tertian forms have much more active movements than the quartan.

3. *Differences in the behavior of the parasite towards the substance of the red corpuscles.* The tertian parasite discolors the red cell much more rapidly and decidedly than does the quartan. Moreover, while the cells invaded by the quartan parasite either preserve their normal size or tend to become smaller, those invaded by the tertian become swollen and tend to grow larger than the normal red cells.

4. *Differences in the morphological characters.* The quartan parasites have better defined and clearer outlines than the tertian. The pigment granulations of the tertian are extremely fine; those in the quartan hæmamœba are larger.

The sporulating forms exhibit some differences consisting in the number of bodies resulting from fission (gymnospores), which average fifteen to twenty in the tertian parasites, six to twelve in the quartan; and in the size of the individual bodies, which is larger in the quartan. Moreover, within each spore resulting from the segmentation of the quartan amœba we see a central shining sphere which represents the so-called *nucleolus* or *nucleoliform body*, while this is not of constant occurrence in the tertian gymnospore.

The differences between the parasites of the *ordinary tertian* and those of the *summer-autumn tertian* (estivoautumnal parasites) are very marked, so that the differential diagnosis is always easy.

These differences relate to:

1. *The size of the parasite.* The parasites of the estival tertian, at the same stage of development, are always smaller than those of the ordinary tertian.

2. *The appearance of the parasitic forms.* The estival parasites, in their first phase of life, long preserve the property of taking on the characteristic annular form in fresh preparations; similar rings are seen in the ordinary tertian but rarely, and they are never seen at an advanced stage of development. Moreover, the annular and discoid forms of the estivoautumnal parasite have more distinct outlines, and stand out more conspicuously against the background of the red cell than do the corresponding forms of the ordinary tertian.

3. *The characteristics of the pigment.* In the ordinary tertian the pigment is abundant and nearly always motile; in the parasites of estival tertian it is in very fine granules, arranged for the most part upon the extreme margin of the amœboid body, and rarely motile.

4. *The alterations produced in the invaded red corpuscles.* These swell with great rapidity in the ordinary tertian, while in the estival tertian they tend to become smaller and to shrivel, the color of the hæmoglobin becoming deeper than usual.

5. *Fission Forms.* Fission is accomplished by a similar process in the two forms of tertian, but the completely sporulated forms are usually much larger in the ordinary tertian, and the individual gymnospores are larger than in the estival parasites.

6. *The forms which begin in man the life cycle which is continued in the mosquito.* These forms are represented in the summer-autumn parasites (estival tertian) by the characteristic crescents, in the ordinary tertian by the large, round, pigmented bodies which have already been described.

Finally the flagellated bodies of simple tertian usually possess a larger number of flagella than do those of crescent origin.

There are other differences relating to some biological properties, as, for instance, the distribution of the parasitic forms in the circulation, and the pathogenic action on man, which we shall take up later.

These differences between the estivoautumnal, the tertian, and the quartan parasites have, since the studies pursued in Italy, been recognized by nearly all investigators who have taken up the subject, so that at the present time no doubt can be cast upon the possibility of distinguishing these three species by a microscopical examination alone.

B. *The results of the injection of malarial blood in man* have greatly contributed to the establishment of the opinion that we are dealing with distinct species, not intertransformable. These experiments show that when blood containing one kind of parasite only, as for instance the quartan or the tertian, is injected under the skin or into the veins of a healthy person, parasites identical with those injected will be developed, and a fever similar to that in the person from whom the blood was taken will be caused. For reasons easy to understand, inoculation experiments are not numerous, but all which have been carried out carefully have given results favorable to the theory of distinct species.

Of inoculations with blood containing quartan parasites, which have reproduced a fever with the same parasites in the person inoculated, we have two cases of Gualdi and Antolisei, three of Di Mattei, one of Calandruccio, one of Baccelli, and three of Celli and Santori.

Of experimental tertian infections produced by using blood containing only tertian parasites, we have several cases—two of Antolisei and Angelini, four of Bein, one of Baccelli, and one of Mannaberg.

We can also state that every time blood containing estival parasites has been injected, a fever with the same parasites has been caused in the patient. Gualdi and Antolisei saw one case, Di Mattei two, Celli and Santori three, Bastianelli and Bignami four. Finally Bignami inoculated two persons with estivoautumnal parasites taken from a case of typical estival tertian, and witnessed the appearance of the same clinical type of fever with the parasites.

Among all the inoculation experiments which have been made, we find only two that at first sight appear to be opposed to the theory of distinct species. These relate to two patients of Gualdi and Antolisei, in whom they injected blood with quartan parasites, with the result that one of the patients developed a fever with estivoautumnal, and the other with tertian parasites. But can only two experiments furnish valid arguments in support of the belief that the parasitic forms of quartan can be transformed into estival or tertian forms? Antolisei gives a critical review of these two cases, in which he states that the patients from whom the blood for the experiment was taken had in the last few months suffered from fevers of various types—quartan, tertian, quotidian, and irregular. It is therefore evident that in the blood of these patients there coexisted the germs of three species of malaria, but in varying quantity, so that the existence of a mixed infection had escaped the notice of the observers and they supposed that they were using the blood of a person with pure quartan for the inoculation. In fact, the same investigators in subsequent experiments in which they used the blood of patients with a recent and pure infection always obtained the reproduction of the same form in the person inoculated.

C. *The intimate relation between the three parasitic species and the clinical forms of malaria* has been recognized by the majority of recent investigators. Each species of malarial parasite differs from the others in respect to the pathogenic action upon man, as may be seen in the description of the various species of malaria. (See The Febrile Attack, in the section on General Pathology, and Classification of Fevers, in the section on Symptomatology.)

The supporters of the doctrine of polymorphism make much of the fact that it is not infrequent to see the same patient affected by different types of fever with different species of parasite. But this merely proves that the parasites and the various febrile types can succeed each other alternately in the same patient, and does not in the least demonstrate that they are transformed into each other.

Just as a clinician, in the present state of our knowledge, would certainly not say, when a quartan succeeds a tertian in the same person, that it is the same fever in which the intervals of apyrexia have become a little longer, so a parasitologist could not hold that the parasites of quartan have been transformed into those of tertian infection.

These facts only prove that in mixed infections given by two species of malarial parasite, the two infections tend rather to succeed each other than to coexist. This is shown by several interesting experiments of Di Mattei, who, having inoculated quartan parasites into a patient who had crescent forms in his blood, saw the crescent infection disappear and the quartan develop; and, on the other hand, upon inoculating a quartan patient with semilunar blood he saw the quartan disappear and an estival infection develop.

To explain the succession of febrile types and of the various parasites in the same person, we must also remember that mixed infections due to two kinds of parasite may coexist, while only one of them exhibits the characteristic clinical manifestations. For instance, we frequently see patients with estival tertian who have tertian parasites in the blood in addition to the estivoautumnal parasites. In spite of this, the grave clinical form of estival tertian keeps the first place, and interferes with a recognition of the mixed infection, unless the blood be examined. But as a rule the tertian parasites very soon disappear from the circulating blood, and the estival affection is to all appearances pure; this does not prevent the occurrence of an ordinary tertian in its typical form and with the characteristic parasites in the relapses after several months' interval. This fact has been adduced in support of the idea that the estival parasites could be transformed into the tertian, with a corresponding transformation of the febrile type. But an accurate observation will demonstrate that the infection was a mixed one from the beginning, and that during its course the parasites alternated, each one causing its own special type of fever.

The apparent transformation of the febrile types may therefore be due to the fact that in a mixed infection one of the parasitic species may remain latent for a long while, and then from some cause or other may reappear with its characteristic febrile type. Thus, for instance, an individual who had for several years suffered from malarial fever of various types was received into the hospital with a typical quartan. The quartan having been cured, he took a cold bath, after which he was taken with high and irregular fever, and estivoautumnal parasites were found in the blood.

We shall see presently that the other facts taught by clinical ex-

perience, the geographical distribution of the various kinds of fever, the almost exclusive domination of one species in certain localities, etc., all favor the view that the malarial parasites are divided into determined species that are not intertransformable.

The arguments which we have briefly given are demonstrative of the specific nature of the tertian, quartan, and estivoautumnal parasites. But while those of the tertian and the quartan each represent a species which, wherever tertian or quartan fever exists, occurs with certain determined morphological and biological properties, it is a question whether the same is the case with the estivoautumnal parasites.

In other words, do the parasites which we have described as estivoautumnal represent one individual species, or do they include various species and varieties? This is a question which we asked ourselves at the very beginning of our researches, and which cannot even yet be answered. Considering that in the groups of fevers bound to the biology of this parasite there are two fundamental clinical types, namely, the estivoautumnal tertian, which is the predominant and most important, and the quotidian, we endeavored to ascertain the morphological and biological differences which exist between the parasitic forms found in cases of typical estivoautumnal tertian, and those found in cases of quotidian.

These differences relate to—

1. *The duration of the cycle of development*, which in the quotidian is completed in about twenty-four hours, while in the tertian, as all our own researches and those of others have shown, it is completed in about forty-eight hours.

2. *The pigmentation.* In the adult forms of tertian the pigment is more abundant, and sometimes endowed with oscillatory movements, which are never seen in the quotidian.

3. *The size of the parasite.* This at the same stage of development is greater in the tertian parasites than in the quotidian; even the fission forms are larger in the tertian.

4. *The amœboid movements.* In the tertian the motility is preserved for a longer time; even in the pigmented adult bodies the movements are more active, and the amœba tends to assume various and grotesque shapes from the rapid emission and retraction of the pseudopodia. In the small amœba of the quotidian the movements in the pigmented stage are less active and of shorter duration.

5. *The duration of the various life phases in relation to the febrile cycle.* The duration of the non-pigmented amœboid form is very long, and may even go beyond twenty-four hours. Moreover, the forms of the young generation in the summer tertian usually appear

in the blood several hours after the beginning of the attack, much later, in other words, than those of the quotidian.

In spite of these differences the resemblances are so great as to render a differential diagnosis very difficult; all these parasites affect the red blood cells in the same way, all possess a life phase represented by the crescent forms. And therefore we ask, are these parasitic varieties in the true sense of the word, or is it one parasite which exhibits great variability in the duration of its development, so that between the two extremes—twenty-four hours (quotidian) and forty-eight hours (tertian)—these are all the intermediate grades? We also note that if one held to this belief it would be easy enough to ascribe the morphological differences to the varied duration of the life cycles.

While affirming with emphasis that the question cannot be decisively answered, we have declared ourselves as leaning towards the belief that the parasites found in summer tertian and those of the quotidian represent closely related varieties of the same parasite. The majority of authors, however, who studied the problem after we had suggested it, while recognizing the existence of an estival quotidian caused by the estivoautumnal group of parasites, held to the second theory formulated by us.

We must recognize the fact that the question remains at the present time just as we first propounded it, in spite of the parasitological and morphological data which we possess. But since we cannot exclude the fact that parasites morphologically so similar that they cannot easily be distinguished from each other represent distinct varieties or species, we hold that the question should at the present day be discussed upon clinical and epidemiological grounds. Now the clinical and epidemiological data, unless we are greatly mistaken, lead to the supposition that the so-called estivoautumnal parasitic *species* includes several *varieties*. But of this we shall treat in reference to the classification of the fevers and to the hæmoglobinuria of malarial patients.

The parasite which completes its whole life cycle without becoming pigmented (Hæmamœba immaculata) probably represents a species or variety by itself of the estivoautumnal parasites. We have already given the reasons why, even in relation to these parasites, the question cannot be definitely answered in the present condition of our knowledge.

The opinion that the malarial parasites represent distinct species receives support from the new facts learned in the most recent studies on the life cycle of the malarial parasite in mosquitos. These researches, although as yet incomplete, permit us to dwell upon the question of the difference of species only with reference to the estivo-

autumnal and the ordinary tertian parasites. In regard to the life of the quartan parasites in the mosquito we do not as yet possess a sufficient number of observations.

In the anophelic life cycle, the tertian sporozoon, according to Bignami and Bastianelli, is to be distinguished from those of crescent origin by the morphological characters already given; the young bodies chiefly distinguished by the form of the sporozoa and the character of the pigment; the forms undergoing development by the size of the little bodies successively produced by the division of the nucleus. The adult forms with sporozoites are distinguished in general by the size and the disposition of the so-called "residua of segmentation" (nucleus de reliquat).

Experimental research leads to the same conclusion. In fact, if we nourish mosquitos with blood containing crescents, and after these have completed their whole life cycle up to the infection of the salivary glands with sporozoites, cause the same mosquitos to bite a healthy person, an estivoautumnal fever will develop. This experiment has been made by Bastianelli and Bignami in the winter, a season in which the anopheles taken in a free state did not give estival fevers, but when they produced a fever at all, caused the ordinary tertian. The anopheles nourished on crescent blood in the Hospital of Santo Spirito, were kept at a temperature of about 30° C. (86° F.) in order that the crescent forms might develop completely as they do in the summer in a free state.

From the results obtained, Bignami and Bastianelli hold that the distinction between the species of malarial parasites is maintained unaltered in the mosquito.

The Hæmosporidia of Birds and Mammals.

The first uncertain reports in regard to the blood parasites belonging to the group of the hæmosporidia slightly preceded the discovery of the malarial parasite. Writers quote the observation of Ray Lankester, who saw in the blood of frogs small, vermicular bodies free in the plasma (1871), and that of Butschli (1871), who stated that he had often seen within the red blood cells of frogs special bodies situated by the side of the nucleus, which, as was later shown, represented the endocellular stage of the free organism seen by Lankester. Later Gaule (1880), knowing nothing of these observations, described in the red cells of frogs his "vermicules," which he believed to be degenerative forms, while shortly afterwards Ray Lankester affirmed their parasitic nature (quoted by L. Opie).

As we see, therefore, there were no distinct notions regarding the

parasites of the red corpuscles when Marchiafava and Celli published their first researches upholding the theory of the endoglobular parasitism of malaria, so that these authors were obliged to search for analogous facts in support of their view among the parasitic diseases of certain plants. They quoted the works of Woronin, which show that some diseases of certain Cruciferæ, especially the Brassicæ, are produced by an endocellular organism which acts in the plant cell as does the malarial parasite in the red blood cell. They also recalled the researches of Zopf, by which it was shown that the vegetative cells of the *Coleocetaceæ* are sometimes invaded by a parasite which he called the *Aphelidium deformans*. The spores of this parasite penetrate into the interior of the cell, and are converted into amœbæ, which are nourished at the expense of the plasma, the nucleus, and the chlorophyll; this latter is converted into a brown mass, which collects at the centre of the parasite during the period of sporulation.

Soon after were published the first observations of Danilewsky (1886) upon the parasites of lizards, turtles, and birds, with which begins the comparative parasitology of the red cell. These were followed by numerous researches, which have continued up to the present time, from which we learn that even in mammals there are parasites of the red blood corpuscles. Thus it has been demonstrated that the parasites of human malaria, which at first appeared to be the sole representatives of a non-bacteriological infection of the red cell, were part of an extensive group of beings, whose numbers are continually being added to.

In this connection we will confine ourselves to the mention of certain facts in relation to these hæmosporidia of various animals, the study of which has exercised an influence upon the development of the theory of human malaria, or which are of importance in the comparative pathology of the blood. A systematic description of all the known forms in the various species of animals would take us beyond the limits of this work; we will therefore merely give a few of the fundamental facts regarding the hæmosporidia of birds and mammals.

Hæmosporidia of Birds.

Danilewsky was the first to observe the great resemblance between the parasites of the red cells discovered by him in birds and those of human malaria, comparing the elongated parasitic forms of birds to the crescent forms in man, the pigmented round bodies to the pigmented bodies of human malaria, the flagellated bodies of birds to those of man; and struck by the resemblances he queries whether all these organisms are identical or not, and decides in the affirmative.

Later Grassi and Feletti distinguished two types of organism in sparrows and pigeons, one type represented by long parasites curved into crescent shape, already described by Danilewsky, the other by a smaller parasite of varying form, usually situated at the extremity of the red cell. They thought the first form similar to the crescents of human malaria, and hence included the two organisms in one genus, the *Laverania*, distinguishing them by the special names of *Laverania Danilewskyi* (in birds) and *Laverania malariæ* (in man). The second form they held to be very similar to the amœboid forms of human malaria; they established in them the genus *Hæmamœba*, which included two species, commonly found in birds: *H. subpræcox* (in *Alauda arvensis*, *Athene noctua*, *Passer hispaniolensis*, etc.), and *H. relicta* (in *Passer hispaniolensis*, *Alauda arvensis*, etc.), to which they added a new species, found in one case only, *H. immaculata*, a species which does not occasion the formation of black pigment as do the others. These hæmamœbæ of birds differ from the species pathogenic in man, because even in the young stages they have no recognizable amœboid movements.

Celli and Sanfelice also endeavored to trace the analogy between the hæmatozoa of birds and those of man, and took as the basis of their classification the rapidity with which these organisms complete their cycle of development. They therefore distinguished: (*a*) parasites of slow development, which reproduce themselves after about eight days of life, and correspond morphologically to Danilewsky's crescent bodies in birds; (*b*) parasites with accelerated development; and (*c*) parasites with rapid development.

They were not able accurately to determine the life cycle of these last two varieties. Morphologically they correspond to the forms of the genus *Hæmamœba* of Grassi and Feletti. The variety *a* differs from *b* in the large size of the fission forms, which in the variety with rapid development are smaller and give rise to fewer gymnospores.

The variety *a* according to Celli and Sanfelice corresponds to the quartan forms in man; *b* to the tertian parasites, and the third variety, *c*, to the estivoautumnal parasites. But while emphasizing these resemblances, they carefully abstain from asserting that there is an identity of species. Indeed, never having succeeded in transmitting the infection from one bird to another of a different species they are inclined to think that, in spite of the marked morphological resemblances between the parasites of different birds, the forms are really distinct, and that each species of bird has its proper parasite which can live in that species alone.

More recently still we have the researches of Labbé, who from the zoological point of view studied the parasite in the blood of verte-

brates. In the birds he again described the two forms seen by Grassi and Feletti and other observers following them. To the small parasites of irregular shape he gave the name of *Proteosoma*, and to the elongated forms the name of *Halteridium*. Thus he maintains the distinction into two genera, as do Grassi and Feletti, from whom he differs in describing one species only for each genus.

The generic names proposed by Labbé have been adopted by many subsequent observers. They possess the advantage of not conveying the idea of a correspondence between certain parasitic forms of man and those of birds. Evidently there is no reason for approximating the *Halteridium Danilewskyi* (Laverania Danilewskyi of Grassi and Feletti) and the crescent forms of man; the latter do not represent a special parasite, but the gamete of an amœboid parasite (estivoautumnal species).

We will describe briefly the fundamental characteristics of these two types of organism.

Roundish parasites of irregular shape (proteosoma of Labbé). The young forms are represented by a roundish, endoglobular body, with difficulty distinguishable from a vacuole in fresh preparations, the more so that at a high temperature it is impossible to see amœboid movements. Increasing in size, the parasite becomes pigmented with black granules, and places itself at one end of the cell; at the same time the nucleus of the cell leaves its longitudinal and median position, and usually places itself at the opposite end of the cell, at or almost at right angles with the long axis of the cell. ' Multiplication, which occurs just as it does in the parasites of man, is observed in the circulating blood. The parasite takes on a round shape, the pigment collects in a block at the centre, and the parasitic body divides into a number of small bodies.

The size of a body in segmentation is usually about half that of a red cell, but some are smaller. From the standpoint of this difference, Celli and Sanfelice distinguish two varieties of parasite, while Labbé and Opie, having always found them together, consider them to be one variety only.

It would appear that these parasites develop in regular groups, the individual members of which are all at about the same stage of growth, as in the parasites of human malaria. It has not, however, been found possible to establish the duration of their life cycle.

Finally, in addition to the bodies which end in segmentation, there are found larger pigmented bodies, with disseminated pigment, which do not multiply, and which Celli and Sanfelice have considered to be analogous with the crescents. Opie holds them to be sterile forms. As some of these bodies develop flagella precisely similar to

those of human malaria, it is to be supposed that they have the same biological significance as the crescents (gametes).

Parasites with an elongated form (Halteridium of Labbé). The adult forms are found by the side of the nucleus, in the direction of the greater axis of the red cell, their two ends being curved around the nucleus.

The young forms are similar to those of the proteosoma, and quickly become pigmented; they then develop by lengthening, and when their greatest axis is longer than that of the nucleus of the red cell, they become curved in crescent shape. Multiplication, it would appear, does not occur in the circulating blood. Labbé has seen segmenting bodies in the bone marrow and in the spleen.

In birds containing many elongated parasites it is easy to witness the process of flagellation in the preparations, the flagellated bodies indeed being usually numerous. It is in these organisms that MacCallum witnessed the phenomena of fecundation—in other words, he saw a flagellum penetrate into a round pigmented body, which shortly afterwards took on the appearance and movements of a vermiculus.

Di Mattei devoted himself to a study of the pathogenic action of these parasites in pigeons especially. He found that the infected pigeons had the same temperature as the healthy ones, or if there was any difference, that the temperature was several tenths of a degree Centigrade lower. Quinine did not exert any perceptible action.

Danilewsky speaks of acute and chronic infections in birds, the acute being determined by the proteosoma, and accompanied by high temperature and the signs of disease, the chronic caused by the halteridium.

As to the *epidemiology* of these hæmatozoic infections of birds, we know in the first place that we may find them infected in localities where there is no malaria, and healthy in malarial regions. Studying in particular the infections of pigeons, Di Mattei has noticed that they are most often infected in the summer, and more in low than in high latitudes. Still the influence of locality, which is so manifest in human malaria, does not appear to play a great part. The association of infected with healthy pigeons does not seem to disseminate the disease.

These data, added to those previously given, positively exclude the identity of human hæmatozoa with those of birds, formerly upheld by Danilewsky because of the zoological and pathogenic resemblances. Finally experimentation has demonstrated that the inoculation of malarial human blood into a pigeon, or that of an infected bird in man, gives negative results.

Some hæmatozoa, in addition to the life cycle completed in birds,

have a second, which is completed in an insect. This second cycle has been studied by R. Ross in the proteosoma of sparrows in the gray mosquito (Culex pipiens), and it is the first cycle of a hæmosporidium in the body of a mosquito which was ever studied. Ross found that in gray mosquitos nourished by the blood of birds infected with proteosoma, there were special pigmented cells in the walls of the stomach; these cells were not found in mosquitos of the same species nourished with the blood of healthy birds. The pigmented cells which originate from the proteosoma, after acquiring a capsule, develop progressively in the stomach walls of the mosquito. Within the capsule is formed an enormous number of filiform bodies which become free when the capsule breaks, and accumulate within the cells of the salivary glands. This collection is found in mosquitos which have been nourished with the blood of proteosoma for seven to eight days. When the mosquitos contain such filaments (germinal rods) in the salivary glands, they are capable of infecting healthy birds by puncture.

This biological cycle, which Ross interprets as the development of a *coccidium* (Proteosoma coccidia) is identical with that studied in Rome in the parasites of human malaria in mosquitos of the genus Anopheles.

Hæmosporidia of Mammals.

The study of the endoglobular parasites of *Texas fever* (hæmoglobinuria, hæmatinuria, or malaria of cattle) has been of great value in elucidating several fundamental questions concerning the biology of the hæmosporidia.

It is a disease characterized by fever, acute anæmia, and in grave cases by hæmoglobinuria, and is found especially in some southern countries, where it chiefly attacks imported cattle which have not become acclimated. The destruction of the red cells is due to an organism discovered in 1889 by Theobald Smith, an organism which lives in the red cells and belongs to the Protozoa, the Pyrosoma bigeminum.

Celli and Santori, who have studied this disease in the cattle of the Roman Campagna, distinguish two forms of the parasite: one which displaces itself *in toto* within the red cell, another which possesses amœboid movements. Those of the first form measure from 1 to 1.5 μ and are round, ovoid, or pyriform; they are isolated or united by twos or threes in the same red cell, and move in one mass within the cell. Those of the second are two or three times larger than the first, but being more transparent, are less easily seen. The amœboid

movements may be very active. These organisms have a marked resemblance to the plasmodia of human malaria.

The disposition within the same corpuscle of these bodies in pairs (whence the name given by the discoverer) corresponds to a phase of life in these beings the biological significance of which has not as yet been well determined. In addition, free forms are found within the plasma.

According to Sidney Hunt, the forms of reproduction of these organisms are easily found within the capillaries of the myocardium in advanced cases, and occur as long bodies more or less semilunar or spheroidal in shape, within which the young pyrosomata are formed by endogenous division.

Of great influence in the development of our knowledge in regard to human malaria have been the interesting researches of Smith and Kilborne concerning the mechanism of infection of healthy cattle with Texas fever.

These authors have demonstrated beyond all question that the disease is transmitted by means of a special tick (Boöphilus bovis Riley). They have in fact proved: (1) that a diseased animal from which all the ticks are carefully removed does not infect a healthy animal; (2) that fields may be infected by carrying to them ticks taken from diseased cattle; (3) that young ticks born of mothers nourished upon diseased cattle produce Texas fever when they are placed on susceptible cattle.

This last experimental fact has recently been confirmed by R. Koch, who studied the same disease in German East Africa.

From these researches we conclude that the parasite of Texas fever is inoculated into cattle by a tick, and that the tick is necessary for the transmission of the disease; in fact, by removing the ticks we suppress the contagion. It is to be noted that this was the first example demonstrated of a disease caused by a parasite in the red blood cell, which is inoculated by means of the puncture of an insect. This fact could not but strengthen the hypothesis that an analogous mechanism exists in the case of hæmosporidia in general, and of the parasites of malaria in particular.

Besides the *Pyrosoma bigeminum* (*Apiosoma bigeminum*) the structure of which has been studied by Ziemann, who demonstrated in it the presence of nuclear chromatin by the use of Romanowsky's method, very few parasites of the red corpuscle are known in mammals. We would recall the parasites of the ictero-hæmoglobinuric infection of sheep studied by Bonome, an endoglobular parasite found in a dog by Piana and Galli-Valerio, etc. Moreover, Koch has demonstrated the existence in monkeys of a pigmented parasite very similar to

that of human malaria. And more recently Dionisi has found in bats various species of endoglobular parasites, which are very interesting because of their marked resemblance to those of malarial fever. Thus in one species of bat (*Miniopterus Schreibersii*) caught in the Roman Campagna, he found parasites greatly resembling those of quartan fever, and he found the same in another species—*Vespertilio murinus*—which is common in the neighborhood of Rome. In some individuals of the species *Vesperugo noctula* (Schreb) he found a parasite of the red corpuscle morphologically similar to the æstivoautumnal parasite of man. This parasite has annular, discoid, and rapidly moving amœboid forms, without a trace of black pigment, which are not to be distinguished from young æstivoautumnal plasmodia. If these forms are followed for several days, it will be found that they never become pigmented, and it would seem that they complete their whole life cycle without forming pigment. They may be abundant in the blood, as occurs in pernicious infections in man, and they cause profound anæmia in the animal in which they live, producing a degeneration of the red corpuscles which corresponds to the *anæmic degeneration* of Ehrlich.

We do not yet know precisely by what mechanism the infection of bats occurs; nor do we know whether the parasites found by Dionisi in bats are identical with or only similar to those found in man, the researches into the subject being still under way. Such knowledge as we possess at present regarding the life cycle of these beings, which appears to be different, at least in their duration, from that of human parasites, and reasoning from analogy (for instance, what is known in regard to the hæmatozoa of birds), cause us to suspect that these parasites represent particular species of hæmosporidia proper to bats and differing from the human parasites. But, as we observed before, the subject is still being studied.

ETIOLOGY.

The Mode of Entrance of the Parasite into the Human Organism.

The malarial germs may enter the human organism either (1) directly from the external world, or (2) by subcutaneous or intravenous inoculation of human blood containing the parasites, or, as some believe, (3) by passing through the placenta of a malarious mother to the circulation of the fœtus. The first mode of infection, which represents the natural mechanism by which one "takes" the fever, is manifestly of great scientific and practical importance, because upon

the knowledge of such mechanism is based the whole subject of individual and social prophylaxis. The second manner of infection is solely of scientific importance. Experiments with the inoculation of a healthy man with malarial blood have aided in confirming the belief of the early investigators that the microscopic beings found in the blood of malarial subjects are actually the causal agents of the disease. They have also served to strengthen the doctrine of the plurality of species of the malarial germs, and to facilitate the study of certain questions of pathology, such as the period of incubation, etc. The third way of penetration, which would be the one effective in the etiology of the so-called congenital malaria, has not yet in our belief been shown to occur.

Direct Infection from the Outside World.

From all that we have said regarding the life cycle of the malarial parasites in certain species of mosquitos, it follows manifestly that the natural way of acquiring malaria is by inoculation. If it is admitted that the facts above described concerning the life cycle of the malarial parasites in the Anopheles represent practically the entire biology of these beings outside of man, then we may assert positively that inoculation is the natural mode of infection, and that there can be no other. The conclusion that "man acquires malarial fevers solely through the bites of certain species of gnats by which occurs inoculation of the malarial sporozoites" would be the logical and necessary consequence of such a premise. But, although we cannot positively deny at the present time that the malarial parasites may exist under other forms than those now known to us, nevertheless we maintain the conclusion above set forth which is founded upon a series of facts tending to exclude the possibility that infection may occur in other ways.

This renders necessary the study of the problem "how fevers are caught" independently of the facts which we possess relative to the biology of the parasites within the bodies of the diptera; that is to say, it is necessary to see what clinical and epidemiological experience in malarious regions teaches, and then to set forth in detail the experiments upon which the theory of inoculation is based. Such an exposition is the more necessary since even in these latter days many authors, while recognizing the importance of mosquitos as vehicles for the transportation of the malarial organism, yet refuse to admit that inoculation is the sole mechanism of infection, and incline to the belief that, as Lancisi held, there are multiple channels of ingress of the malarial germs. This opinion indeed is very widely

held. Manson, for example, who held up to within a short time that infection occurs through the water of malarious regions, has naturally been forced to admit recently that it may be brought about through inoculation by mosquitos, yet he still maintains that the malarial germs may enter the organism by either inhalation or ingestion as well.

Up to within a recent period three hypotheses were held regarding the mechanism of infection by the malarial germs: (1) The water theory, (2) the air theory, and (3) the inoculation theory. The third theory, that the malarial germs are inoculated into man through the agency of mosquitos, is the only one which has up to the present time been demonstrated experimentally. But since, as we have said, many still believe that the malarial germs can penetrate into the organism by means of ingestion and of inhalation, it is necessary that, before proceeding to an exposition of the facts which demonstrate conclusively that malaria may be transmitted by certain species of mosquitos, we should examine briefly the arguments and the observations which have been urged against the probability of these two hypotheses.

The Water Theory.—There is an abundance of facts which are cited by authors in support of the hypothesis that man may become infected with malaria by drinking water from marshy places. Without referring to these in detail (they are enumerated in numerous treatises, such as those of Laveran, Sternberg, Mannaberg, and others), we may in general group them into certain classes as follows: 1. It has often been asserted that of certain individuals living in a special locality under otherwise identical conditions, but drinking water from different sources, some were attacked in large proportion by malaria while others were spared by the fever. 2. In certain insalubrious localities it has sufficed to provide a pure water-supply to the inhabitants, water from stagnant pools having previously been used for drinking purposes, to cause the malarial fevers to disappear. 3. In very healthy places the fever might be contracted when water brought from an unhealthy place was used for drinking, and those individuals who were most apt to take the fever under these conditions were the ones who had consumed the greatest amount of the suspected water. 4. Examples have been cited of travellers who, while passing through malarious countries, had succeeded in preserving themselves from the fever by drinking only boiled water, while those who did not take this precaution were attacked in large numbers.

We may note at the outset that many authors, as, for example, Laveran, who was regarded until very recently as the chief advocate

of the water theory, recognize that many of the facts upon which their arguments are based are not wholly above criticism. And, first of all, many of the facts themselves are not definitely established and are rather vaguely stated. In many cases no proof is given that the fevers which followed the ingestion of the alleged unhealthy water were truly malarial. Others of the facts given are open to various interpretations. When, for example, it is affirmed that the intensity of the malaria diminished after a district had been supplied with pure water, we must remember that this effect may have been due to a general improvement in all the hygienic conditions; malaria, we know, retires before the progress of hygiene and civilization. When we hear of individuals living under identical conditions, of whom those acquired malaria in greatest number who were forced to drink water from stagnant pools, we forget that this very fact itself shows that the conditions were not really identical. We cannot exclude the possibility that those who drank the stagnant waters may have been exposed more than the others to the occasional causes of malaria or offered less resistance to the germs of the disease; nor can we exclude the possibility that the drinking of stagnant water may facilitate the development of the parasites which have already, in some manner, penetrated into the organism, as we know happens through the influence of poor food, a chilling of the surface, etc.

To the facts recited, for a detailed criticism of which the reader is referred to the works of Celli and of Zeri, may be opposed the results of experience in the Roman Campagna. Many places, in fact, are known in the environs of Rome which are exceedingly malarious, yet in which the inhabitants drink the same excellent waters as those supplied to the city itself. In other places, Ostia for example, good drinking-water has been introduced with no improvement in respect of malaria. A study of the epidemic of malaria at Sinigallia, of which we shall speak later, leads us to deny that water is of any importance as a vehicle of infection. In other malarious regions careful observers, such as L. Martin in Sumatra, Schellong in New Guinea, Werner in Samara, Russia, and Rupert Norton in America, have arrived at the same conclusion.

Evidently for a solution of the problem it was necessary to resort to experiment, to determine—namely, whether water taken from malarious to absolutely healthy districts could convey infection to healthy individuals drinking it. Experiments of this nature have been carried out in Rome by Celli and Zeri, in Sicily by Brancaleone and Salomone Marino. Celli had healthy individuals in the Santo Spirito Hospital drink for several days water collected in the Pontine marshes and from stagnant pools in the environs of Rome, but with negative

results. Zeri, at the suggestion of Celli, carried out three series of experiments: 1. He had nine persons drink for from five to twenty days 1.5 to 3 litres daily of water taken from malarious places, so that each person ingested from 10 to 60 litres (quarts) of the water. 2. He sprayed the mucous membranes of the respiratory apparatus with marsh water by means of an ordinary compressed-air atomizer, experimenting in this way on sixteen individuals. 3. In five persons he experimented with rectal injections of water from malarious regions. In none of these experiments was he successful in producing an attack of malaria.

A single positive fact favoring the water-borne theory has been reported by R. Ross from India. Led by the hypothesis previously mentioned of Laveran and Manson (according to which the mosquitos, having taken in human blood charged with malarial parasites, go to deposit their eggs in water and there die, whence the infection of the water itself) he had a person drink water in which there were dead mosquitos containing malarial parasites. Eleven days later the subject of the experiment had an attack of fever which lasted three days and ceased spontaneously, no relapse following. In the blood of the patient Ross declared that he found many annular forms of the plasmodium. But in other individuals upon whom Ross repeated the same experiment there followed no fever which could certainly be called malarial. This, of course, takes away all value from the results of the first experiment, and all the more as the course of the fever occurring then was so unlike that of ordinary malarial infection that one is justified in doubting the exactness of the observation.

We may thus conclude that all attempts to cause a malarial fever experimentally by means of the ingestion of the waters of swamps have failed.

The Air Theory.—This theory of malarial infection was held up to very recent times by the majority of physicians and by the almost universal consensus of the inhabitants of malarious regions. The partisans of this hypothesis maintain that the free life of the parasite is passed in the soil or in the water of marshy places, whence it passes into the air and infects man through the channel of the respiratory organs. Numerous researches have been made with the aim of discovering the germs in the so-called malarial materials, but all without result. Among the most recent of these experiments we find those of Grassi and Calandruccio, who held, some years ago, as a definite fact that the malarial parasites were rhizopods or forms related to them; they therefore sought for them among the members of this group which are found, in their free existence, in "materia malarica," such as uncultivated fields, made land, rice-fields, etc.

They formulated the hypothesis that the malarial parasites were to be found in the genus Amœba, in its wide sense, and they assumed that certain amœbæ, living a non-parasitic existence, became encysted, were carried in the air, and so entered the body of man, there developing and taking on characters somewhat different from those of their ancestors in the non-parasitic life. But since this assumption is overthrown by the results of modern researches into the biology of the malarial parasite, it can evidently not be cited in support of the air-borne theory of malaria.

Let us see now, in the absence of direct observations, whether this hypothesis accords with the epidemiological and clinical data in our possession. Authors have found much difficulty in explaining by the air-borne theory certain of the best attested epidemiological data of malaria. It does not in fact explain satisfactorily how the germs enter the air from the soil to which latter epidemiologists in general assign the origin of the miasm; nor does it explain why malaria is not carried by winds or at least is not notably so carried; nor, again, does it explain why the charge of malaria in the atmosphere varies so markedly at different hours of the day.

It might be thought that the germs could rise into the air from the soil along with dust. But to this it would be objected that malaria does not act like a disease caused by inhalation of dust; and furthermore that the days of greatest danger are windless, when less dust rises, and especially on the warm still days following a rain in which no dust rises from the damp earth. If it were alleged, on the other hand, that the germs pass into the air from humid soil, then it would be necessary to assume that something occurs with great facility and as a rule in the case of malarial germs which, in that of ordinary bacteria, has never been satisfactorily shown to take place—an altogether arbitrary supposition.

As to the action of the wind, it was long ago asserted by Hirsch and more recently (with a wealth of argument) by Tommasi-Crudeli, that the wind transports malaria only very short distances, if at all, and that practically it plays no part in the diffusion of the disease. Classical examples of this fact are found in Latium. If the emanations from the Pontine marshes were the cause of the malarial fevers in the Roman Campagna, as Lancisi believed, then it is impossible to understand why the cities of Velletri, Genzano, Ariccia, Albano, etc., which lie between Rome and the marshes, and ought to receive first and in greater concentration the noxious emanations transported by the wind, should be entirely free from malaria. If malaria could be transported to a considerable distance by the winds we cannot understand its presence in strictly circumscribed regions, examples

of which limitation are to be found in Italy and many other parts of Europe and in America.

Furthermore, Tommasi-Crudeli called special attention to the well-known fact that malaria rises but a short distance above the ground. Experience has taught the inhabitants of the Pontine marshes to sleep at night, during the fever season, on platforms raised on poles four or five metres (thirteen to sixteen feet) high. The same practice is followed in certain parts of Greece. Passing over other facts leading to the same conclusion we may note that Tommasi-Crudeli explains the notable differences that exist in regard to malaria between Norma, Sermoneta, and Sezze, cities lying above the Pontine marshes, by the fact that the germs do not rise far above the plain.

If it be conceded that the germs are in the air, how can we explain these facts of observation, to which many similar examples might be added? The sea breeze which blows in Rome in the summer does not bring danger, yet it passes over all the numerous foci of malaria in the western half of the Campagna and over all the swamps on the coast. But it is not, writes Tommasi-Crudeli, that this breeze does not carry malaria in the direction of Rome, for it does carry it and in large amount; but it carries it while acting at the same time as a ventilator—that is to say, it scatters the germs in every direction, although it is a current of air of very slight velocity. To this we may reply that it is not easy to convince one's self that a current of air, carrying every day, as Tommasi-Crudeli holds, a large quantity of malarial germs, does not fill the city with malaria, and the suspicion arises naturally that the argument does not start from correct premises, and that in fact the sea breeze does not transport any malarial germs at all.

Without entering upon a more minute critical examination, such as has been made by Bignami, we may say that the air-borne theory does not permit of a satisfactory explanation by epidemiological data; and despite the most ingenious attempts at an explanation, how is it possible to conceive that the winds do not transport the germs of malaria if these are present in the air? How can we explain the fact, which has been repeatedly observed, that the crews of ships lying off the most insalubrious coasts escape, only those men being attacked whose duties compel them to pass a large part of the time on shore? Why are the evening and the night hours the most dangerous? And how are we to explain on this theory the great difference as regards the danger of infection between waking and sleeping in a malarious region?

The Inoculation Theory.—The insurmountable difficulties which

we encounter in admitting either of the above-mentioned theories, the air-borne or the water-borne, naturally lead us to think of some other mechanism by which the malarial germs may gain entrance into the human body, and more especially of inoculation. We are driven to this hypothesis partly by the exclusion of the two preceding ones; partly by the fact that subcutaneous or intravenous injection of malarial blood is the only means by which hitherto malarial fever has been produced experimentally, and partly by the analogy of human malaria (in a parasitological sense) to Texas fever, which, as Smith and Kilborne have demonstrated, is inoculated into the animals by a species of tick.

We have already recounted the history of this theory. Bignami, in an article published in 1896, demonstrated its probability, holding that many facts which are difficult of explanation by the air-borne theory are readily and satisfactorily explained by this hypothesis. Thus, admitting that malaria in man is the result of inoculation by mosquitos, it is not difficult to explain why it is practically not carried by the wind; it is also easy to understand why the danger of acquiring malaria is greatest in the evening and the night. We see at once why the infection does not rise far above the ground. We comprehend readily the danger of sleeping in malarious districts; and finally this theory explains perfectly the well-known prophylactic efficacy of mosquito-nets in regions where malaria prevails. All this indeed accords exactly with what we know of the habits of mosquitos in malarious countries, which sting especially at evening and during the night, do not fly far from marshy places where the proper conditions of their existence prevail, are in hiding during the day out of the way of the winds, are most numerous in places where malaria prevails, disappear from places where works of sanitation have removed the conditions necessary to their existence, do not fly to any great height above the ground, etc. This theory also explains the efficacy of the prophylactic measures adopted, as the result of experience, by the inhabitants of malarious regions. Many of these precautions taken against the fever seem really to be taken against the attacks of mosquitos.

Experimental Infection by the Stings of Mosquitos.

A consideration of these facts led Bignami to the conclusion that malaria acts like a disease inoculated through the stings of mosquitos. In order to verify this hypothesis experimentally it was necessary to cause healthy men living in a positively non-malarious district to be bitten by mosquitos transported from a place where malaria prevailed. After some fruitless attempts, these experiments,

conducted with the utmost scientific rigor during the past year, have given positive results, the precaution being taken to capture adult mosquitos in a pronouncedly malarious region somewhat late in the season, as the number of infected insects is much greater at that time than at the beginning of the season of malarial prevalence. The first experiment with absolutely positive results was carried out by Bignami in the person of one Abele Sola, an inmate of the Santo Spirito Hospital for the past six years, who suffered from a nervous malady but had never had malaria. He offered himself voluntarily as a subject of the experiment. This was carried out by liberating in a suitable room provided with mosquito-bars mosquitos brought from Maccarese, a marshy place with an evil, but deserved, reputation for the intensity of its fevers. We will quote here Bignami's description of his experiments.

Experiment No. 1.—"Sola slept in the room [in which the infected mosquitos had been liberated] from September 26th to the end of October, 1898. During the latter part of October the patient complained of malaise and headache. On the afternoon of October 31st he had a slight elevation of temperature to 37.2° C. (99° F.). On November 1st at about 3 P.M. he was taken with a severe chill which lasted until five o'clock, the temperature rising rapidly to above 39° C. (102.2° F.). Between nine and ten o'clock a feeling of cold was again experienced. The fever continued all night, falling in the early morning (November 2d) to 38.2° C. (100.8° F.), and rising again that evening to 39.3° C. (102.7° F.). The patient was restless and complained of very severe headache, but there were no grave symptoms. In the night, about eleven o'clock, he had another chill of short duration. During this night the temperature remained above 39° C. (102.2° F.), and on the morning of November 3d rose above 40.4° C. (104.7° F.), the patient being very restless and complaining of much suffering. The fever broke in the afternoon with a gentle perspiration.

"At a quarter after five in the afternoon a hypodermic injection of 1 gm. (gr. xv.) of quinine was given and repeated in the night. The fever fell and at 8 A.M., on November 4th, the temperature was 36.7° C. (98° F.). The administration of quinine was continued during the following days, the patient continuing to have slight elevations of temperature which did not reach 38° C. (100.4° F.) except once on November 6th. From November 7th onward the patient was entirely without fever and rapidly regained his appetite and strength.

"An examination of the blood made with the greatest care on November 2d gave negative results, no malarial parasites being found. On the morning of November 3d a few young annular para-

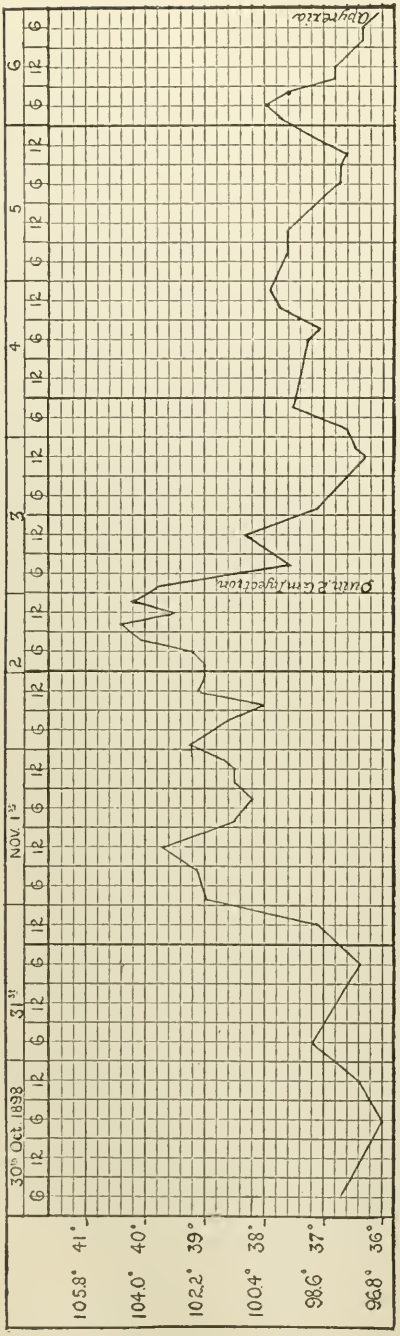


CHART A.—Experimental Malarial Fever. (Bignami.)

sites, motile and discoid, without pigment, and presenting the characteristic appearance of the parasites of estival fever, were found. These forms increased in number during the day, and were quite numerous during the afternoon hours. In some there was a beginning pigmentation at the border consisting of very fine granules of pigment. Before the quinine was given, dried preparations were made which were then fixed in absolute alcohol and stained with hæmatoxylin and eosin after Romanowsky's method. In these preparations the parasites appeared in the characteristic annular form with chromatin stained in the typical manner.

"We see, therefore, that there was produced experimentally in Sola a grave malarial fever with a temperature curve such as is frequently seen in a primary estivoautumnal infection. The fever began briskly, continued with slight remissions from November 1st to 3d, and began to fall on the evening of the latter day when the specific remedy was administered. The parasites found in the blood belonged to the estivoautumnal species. Taking into account these findings and the course of the fever, we believe that the continued curve of the latter, had it not been for the therapeutic intervention, would have become transformed into that of an estival tertian fever (see Chart A).

"This is perhaps the first time that, in the primary estival infection acquired in the natural way, examinations of the blood have been made from the beginning of the fever. We find that after about forty hours the parasites begin to be found, at first in small numbers, but rapidly becoming more and more numerous. According to the opinion of all who followed this experiment it was conducted in such a way as to silence all objections. Sola is a robust individual, notwithstanding his nervous malady, who has never in his life had a malarial fever, and who has not been outside of the Santo Spirito Hospital for six years. The room where the experiment was conducted was an annex of the San Carlo ward in which, within the memory of the hospital physicians, there has never been an autochthonous case of malarial fever, nor has there ever been any malaria in the neighboring houses.

"The objection that the results of the experiment are not conclusive because it was conducted in Rome, where some believe that there is always danger of contracting a malarial fever, can have an apparent value only in the minds of those who do not know anything about Rome and the distribution of malaria in this region. But it is well known to all physicians here that, although there are some centres of malaria in certain portions of the suburbs, the city proper is entirely free from malaria, as long experience has demonstrated, and in no season of the year does one acquire the disease in Rome. Now in a room in the San Carlo ward of the Santo Spirito Hospital Sola acquired a malarial infection produced by estivoautumnal parasites, with a well-marked fever and with symptoms so grave as to call for the prompt administration of quinine. The fever indeed was exactly such a one as is ordinarily caught by laborers in the Roman Campagna in the summer and autumn months, a fever beginning with the typical curve of the estival tertian or sometimes with a continued curve. This identical fever, such as prevails at Maccarese, was taken by Sola in a place where there was of Maccarese neither the water nor the air nor the soil, but the mosquitos alone. We are then forced to the conclusion that this fever was acquired by inoculation by the mosquitos." *

What were the species of mosquitos employed in this experiment? Grassi, who classified them, distinguished three species, Culex penicillaris, C. malariæ, and Anopheles maculipennis, the three species, namely, which, because of the great abundance in which they are found in malarious regions, had already been suspected by him of being carriers of the infection. But this experiment was conducted

* Sola had several relapses following this experimentally induced attack, but each of them was promptly treated with quinine and quickly cured.

in such a way as to make it impossible to determine whether the infection developed in the patient was due to the action of all three of these species of mosquitos, or to two of them, or to only one.

Soon after this some new experiments were undertaken with a single species of mosquito, the Anopheles claviger, caught in the Roman Campagna, the enormous prevalence of this species in the Campagna, especially towards the end of autumn, having been remarked. All the experiments undertaken with this species were successful in their results. These results were announced in various preliminary communications made by Grassi, and Bignami and Bastianelli, and later were published in full detail by the two last named. On account of their importance we reproduce here the account given by Bastianelli and Bignami:

Experiment No. 2.—"N. N——, a robust young man, who has never had malarial fever, but is under treatment at the Santo Spirito Hospital for a nervous trouble, consents to sleep in the mosquito room on the night of November 13th–14th. The day before about one hundred individuals of A. claviger caught at Maccarese were liberated in this room. The subject of the experiment continued to sleep regularly in the room up to December 2d; he entered it at dusk and left it in the morning, passing the day in the general ward. He asserts that he was bitten by the mosquitos every night, and there were always found in the room numbers of A. claviger full of blood. Some of the insects died during this time, but on November 29th there were still found about fifty in good condition.

"The first two days of December the patient was indisposed and had a slight elevation of temperature. On December 3d he had a febrile attack which began with a chill, the temperature rising to a maximum of 39.7° C. (103.5° F.). Similar attacks, each preceded by a chill, were observed on the following days up to December 7th, when 2 gm. (gr. xxx.) of quinine were administered. On December 8th and 9th slight elevations of temperature were observed, but after that none. The patient continued to take quinine for a time and rapidly recovered. The following are the temperature observations in degrees Centigrade from December 3d to 7th:

"December 3d. 4 P.M., 39.8°; 6, 39.7°; 8, 39.7°; 10, 37.5°; 12, 37.7°.

4th. Apyrexia up to 6 A.M.; 8, 37.6°; 10, 38°; 12, 38.9°; 2 P.M., 40.2°; 4, 39.5°; 6, 37.5°; 8, 37.6°; 10, 37.7°; 12, 37.8°.

5th. Apyrexia up to 10 A.M.; 12, 39.5°; 2. P.M., 39.9°; 4, 40.5°; 6, 39.1°; 8, 38.5°; 10, 37.5°.

6th. Apyrexia from the last entry up to 8 A.M.; 10, 39.3°; 12, 40.5°; 2 P.M., 37.8°; 4, 38.7°; 6, 38°; 8, 37.1.°

7th. Apyrexia from last entry up to 10 A.M.; 12, 38.7°; 2 P.M., 38.1°; 4, 38.3°; 6, 37.5°; 8, 37.3°.

"The course of the fever was that of a double tertian. From the first febrile paroxysm tertian parasites were found in the blood, which followed regularly the development in two generations corresponding to the daily attacks of fever. A fact worthy of consideration is that from December 6th, that is to say, four days, or, at the most, five, after the beginning of the disease, the patient had in his blood adult parasitic forms capable of further development in the body of anopheles. And in fact many individuals of A. claviger which had bitten about eleven o'clock on the morning of this day were found later to have the middle intestine infested with tertian parasites."

Experiment No. 3.—"Having heard from a patient in the service of one of the writers in the Santo Spirito Hospital, who was suffering from relapsing quartan fever, that among the laborers in a sand quarry near Tre Fontane there were several who had malarial fever, and especially quartan, we went to the place on December 9th to investigate the report. Upon interrogating the men and making an examination of the blood of the sick we found three cases of quartan and one of tertian. There were also others who said they had fever from time to time but were taking quinine, and we were unable either by their account or by the results of blood examination, which were always negative, to determine with what form of fever they were affected. From the reports, however, of all the inhabitants of the place it may be definitely asserted that there had been no cases of grave (estivoautumnal) fever among the men; indeed, all the men were able to continue at work despite their fever, and left off only for a half a day at a time, or at most for one whole day during the febrile paroxysm, returning immediately after this had subsided. We learned also from the inhabitants that up to within a few days of our visit there had been mosquitos about, but that following a marked fall in the temperature they had disappeared. We succeeded in finding in a room which had been kept closed for several days and in a closet a small number of hibernating mosquitos, all of the species Anopheles claviger; and of these ten or twelve were captured in glass tubes and taken to Santo Spirito.

"Of the mosquitos so taken only seven were utilized in the experiment, which was carried out in a manner different from the preceding. The mosquitos were not set free in a room or under a mosquito

bar, the subject of the experiment then sleeping there, but the glass tube was placed with its open end against the skin and was held fixed there until the mosquito confined in each tube had stung the man. There was here the advantage of knowing precisely the number of mosquitos that had stung the subject and the moment in which each puncture took place, and also of being able to examine later all the mosquitos which had been utilized in the experiment. And so the hibernating insects (A. claviger), regaining their activity in the somewhat elevated temperature of the laboratory, become voracious and they could be seen through the walls of the tube feeding upon their victim.

"A man, A. F——, who had been an inmate of the hospital for several years, but who had never suffered from malarial fever, offered himself voluntarily as a subject of experiment. On December 10th he was bitten by two of the mosquitos which we had caught at Tre Fontane, on the following day by another, and on December 13th by four others; therefore in four days he had received seven stings. Of the seven mosquitos employed in the experiment, three only were examined under the microscope, the four others having been found dead and somewhat dried in the morning and consequently unfit for an examination. In one of the insects some pigmented bodies were found in the middle intestine, but none in the salivary glands. In another, none was seen in the intestine, but instead, one tubule out of four of the salivary glands was found infected. This tubule examined in a fresh specimen was found with the cells of the fundus apparently full of granules of a uniform size and massed together, but it was seen in certain of the cells from which the contents were escaping that the apparent granules were really filaments, thicker in the centre and tapering off at each extremity, and which, disposed in bundles within the salivary cell and presenting their ends to view, looked like granules. In the same preparation, fixed in formalin and alcohol and stained with hæmatoxylin, it could be observed that the cells containing the filaments were enlarged and of a crescent shape with the nucleus pushed to one side. The normal cells, on the other hand, contained a hyaline material and a vesicular nucleus with a nucleolus near the base of the cell. The filaments outside the cells were formed of a thicker median portion strongly stained with hæmatoxylin, and two very fine extremities less strongly stained (sporozoites). The examination of the third insect gave the same result—namely, no pigment bodies in the intestine but the salivary glands infected.

"The same result was obtained on examination of some twenty new specimens of Anopheles claviger caught on December 12th in the same house near Tre Fontane, that is to say, the salivary glands

of each were alone infected. This is readily comprehensible when we remember that at this period the insects had been hibernating for a certain time and were not stinging, whence the absence of pigmented bodies in the intestine while in many the infection of the salivary glands persisted. The positive results of the experiment could have been foretold from this finding. Indeed, on December 29th, after an incubation of from sixteen to nineteen days, A. F—— was taken with fever in the evening. The following morning he was apyretic, but in the blood were found tertian parasites, very near the stage of multiplication, which gave occasion that same day to a febrile attack with a temperature of 39.6° C. (103.3° F.). On December 31st there was another febrile paroxysm, and in the blood tertian parasites of two generations were found; there were also seen some large adult parasites with motile pigment, which became vacuolized (gametes). The patient was treated systematically with quinine and speedily recovered."

Experiment No. 4.—"A patient suffering from a relapsing estivo-autumnal infection, in whose blood were many crescents and round and flagellated bodies, slept from December 10th to 18th in a room in which had been set free about fifty individuals of A. claviger brought from Maccarese. The temperature of the room was maintained at from 18° to 22° C. (64.4° to 71.6° F.). Most of the mosquitos stung the patient and became infected with crescent bodies, and subsequent examination showed in the middle intestine the characteristic forms in process of development. But it was also observed that the mosquitos remaining in the room at the given temperature during the last days of December did not have in the intestine mature sporozoa with sporozoites, but only the growing forms. Evidently at a temperature of from 18° to 22° C. the life cycle of the parasite is completed very slowly. But these same mosquitos, confined for a few days in an incubator at a temperature of 30° C. (86° F.), were found to contain forms of a later development. There were noted, indeed, in the intestine typical capsules filled with sporozoites, and also broken and empty capsules, and in the salivary glands were numerous sporozoites.

"When this fact was noted, three mosquitos of this group were kept in the incubator at 30° C. for two days, and on January 2d they were made to sting a new subject, A. B——, who lent himself knowingly and willingly to the experiment. It is needless to say that this man had never had malarial fever. On January 5th, two of the same mosquitos were made to sting the same person again; who then had been stung in all five times by three specimens of A. claviger.

"After this part of the experiment the three mosquitos were dis-

sected and examined under the microscope, with the following result:

"A. claviger, No. 1.—In the intestine were found very many capsules with sporozoites, and some capsules which had been ruptured and completely emptied of their contents. In the salivary glands were found two infected tubules; in one were seen the cells swollen, of ovoid form, and filled with granules of uniform size; when pressure was made on the preparation there issued a very large number of sporozoites of typical form, uniform in appearance, and all of equal length; in the other tubule were also seen cells containing filiform sporozoites of characteristic appearance.

"A. claviger, No. 2.—In the intestine were very numerous capsules, some still whole and filled with sporozoites, others ruptured and shrunken, containing a granular residuum of a pale yellow color. In some of these ruptured capsules were seen also *brown bodies* of variable size and shape, some elongated, others short and deformed. In the salivary glands all the tubules were infected except one or two. In them were seen cells containing typical sporozoites, cells filled with granules similar to those described in the case of A. claviger No. 1, and cells filled with round hyaline bodies of variable size. In addition there were also found typical filiform sporozoites along the excretory duct of the gland.

"A. claviger, No. 3.—The intestine was filled with mature sporozoa. Many capsules were broken and shrunken and contained a pale yellow detritus; others contained a large central body of granular aspect surrounded by a hyaline halo and without any recognizable structure. These were possibly mature sporozoa in process of degeneration. The salivary glands were not found infected. From the results of this examination we may conclude that of the three specimens of A. claviger employed only two had inoculated the patient with malaria.

"On the evening of January 10th the patient had a sense of heat and a headache, but the temperature was normal. On January 11th, 12th, and 13th there was no fever and the patient felt well. On January 14th, that is, after from nine to twelve days of incubation, there was no fever until eight o'clock in the morning, but then the temperature began to rise rapidly and reached 39.5° C. (103.1° F.) at noon. From this time the fever remained continuous up to January 18th. On January 14th, the temperature fluctuated between 39.5° at noon and 37.5° at 4 P.M. At midnight it was 38.9°; at 2 A.M. on the 15th, 39.5°; at 10 A.M. 37.5°, and at noon 40.4°. On the 16th it varied from 37.2° at 6 A.M. to 40.4° at 4 P.M. The extremes on the 17th were 38.2° at 4 A.M. and 40.4° at 4 P.M. The temperature fell to normal at 6 A.M. of the 18th.

"On January 16th a hypodermic injection of hydrochlorate of quinine 2 gm. (gr. xxx.) was administered, and this was repeated on the following day. The patient was entirely without fever on January 18th, but he continued to take quinine, and, except for very slight elevations of temperature on the evenings of January 19th and 22d, he had no further trouble. Recovery was perfect and rapid.

"On examination of the blood on the morning of January 15th there were found scanty estivoautumnal parasites with very fine pigment granules at the periphery. On January 16th there were found also plasmodia without pigment and with granules in normal red blood corpuscles and in brassy bodies. The parasites disappeared after the exhibition of quinine on January 17th. Thus the infection was rapidly cut short, and no crescent bodies were seen.

"We have in this case a typical example of estivoautumnal infection beginning with a continued fever as is usual in this group of malarial affections. The course of the disease was in every respect identical with that in the first case of malarial fever experimentally induced by the stings of mosquitos (the Sola case), described by Bignami."

With these three new cases of malarial infection experimentally produced by Anopheles claviger alone, the inoculation theory acquires a very strong confirmation. The individuals submitted to experiment have been few in number, but it is to be noted that whenever an attempt was made under appropriate conditions to excite the fever by inoculation it was successful. These last experiments also proved that very few punctures by very few infected mosquitos suffice to give the fever. In the last experiment, indeed, a positive result was obtained with only two infected insects, and it is quite certain that one alone would have been sufficient. And this need cause no astonishment when we think of the enormous number of malarial sporozoites which can be found in the cells of a single tubule of a salivary gland of Anopheles. This answers the objection advanced by many that there are malarious regions in which very few mosquitos are found; account must be taken especially of the species and the number of infected insects.

It may also be asserted that a solitary specimen of Anopheles may infect several men. Indeed we have found sporozoites in the salivary glands and mature sporozoa with sporozoites in the middle intestine in mosquitos which had bitten healthy men and caused in them an attack of malaria. In this case, therefore, even had the Anopheles emptied the entire contents of the salivary glands at each bite, the glands would again be invaded by other sporozoites from the middle intestine, and so the insect would again be capable of inoculating other men with the disease.

In presence of this direct proof we may therefore conclude: Malaria is a disease which is inoculated into man by certain species of mosquitos; and furthermore a critical examination of the air-borne and water-borne hypotheses and the knowledge which we possess of the biology of the malarial parasites outside of man lead us to the well-founded opinion that inoculation is the only mechanism by which the malarial fevers are acquired.

Experimental Infection by the Injection of Malarial Blood.

Malaria is ranked among the miasmatic diseases, among those, that is to say, the pathogenic parasite of which having penetrated into the human body there multiplies, but does not pass from the body into the external world either in the expired air, in the secretions or excretions, or in the products of post-mortem decomposition. Some of the older writers, it is true, believed that malaria could be transmitted in the sweat, but this opinion we now know to have been erroneous. Dochmann (cited by Mannaberg) believed that he had reproduced a malarial fever by inoculation with the contents of an herpetic vesicle occurring on a sufferer from quartan fever, but this experiment has never been repeated and the results lack confirmation. Inoculation with blood containing the malarial parasites will, however, transmit the fever to a healthy man. This fact has been experimentally demonstrated by Gerhardt, who saw a typical intermittent fever reproduced in an inoculated subject, and it has been corroborated by Marchiafava and Celli, who furnished absolute proof of the transmission by finding in the blood of the inoculated person the same parasites which were present in the blood of the patient from whom the material for inoculation was derived.

These experiments were repeated later by many, and by them was obtained a confirmation, as we have already noted, of the doctrine of the multiplicity of species of the malarial parasites; and further, they gave results which were of great utility in the study of the doctrine of incubation. The transmission of the disease occurs equally whether the blood is taken during the apyretic period or during a febrile paroxysm, whether it contains young parasites or those in process of development, or whether it contains sporulation forms. Only the crescent forms, when injected alone, do not transmit the infection, as has been demonstrated by Bastianelli, Bignami, and Thayer, and as can be readily understood when we remember the biological significance of these forms.

In order that the disease be reproduced in the inoculated subject it is not necessary to inject the malarial blood into a vein of the recipient, as has been done in most of the experiments; a subcutaneous

injection is all sufficient. Nor is it necessary to inject several cubic centimetres, as was done especially in the earlier experiments; a fraction of a cubic centimetre will suffice, and even less than one drop, as Bignami has shown. Most of the experiments were made by injecting blood in the natural state, soon after it had been drawn from the patient's vein; but positive results have also been obtained with the injection of defibrinated blood (Celli and Santori), of blood obtained by means of leeches (Bein and Sakharoff), and of red corpuscles separated by centrifugation (Mannaberg). In one case Di Mattei collected the blood from a case of epistaxis occurring in a malarial subject, in a test-tube containing sterilized and distilled water at a temperature of 37° C. (98.6° F.), and injected 4 c.c. (one drachm) of this mixture of equal parts of blood and distilled water; the subject inoculated had a fever fourteen days later. If, however, malarial blood is mixed with an equal quantity of distilled water, the mixture being well shaken, a healthy man may be inoculated with the product, after it has stood about an hour, with impunity (Calandruccio, cited by Grassi and Feletti). If blood rich in parasites is dried at the temperature of the air and then dissolved in a tepid physiological salt solution, an injection of the solution will be innocuous, even when the blood has been left in the dry state for a very short time, as Bignami demonstrated in one case. This same observer has also noted that blood, which is filled with parasites, taken from a patient with pernicious fever may be injected without results, after the administration of large doses of quinine, even though the parasites themselves, examined by Romanowky's method at the very moment of injection, are seen to be perfectly normal in their morphological characters.

The transmission of infection through the injection of malarious blood is possible only from man to man, and then it is extremely rare for the inoculated person not to take the fever, a positive result being the almost constant rule. On the other hand all attempts to induce malaria in various species of animals by injections of blood containing the parasites of human malaria have been uniformly unsuccessful, even when the subjects of the experiment were animals which are subject to infection by hæmatozoa very much resembling those found in man. Celli and Sanfelice have inoculated without success horses, mules, guinea-pigs, rabbits, hedgehogs, bats, pigeons, owls, turtles, lizards, and frogs; Di Mattei, cats and wolves. Various experiments, always negative in their results, have been made on various species of monkeys by Richard, Fischer, Angelini, and Di Mattei; Angelini inoculated a young cynocephalus sphynx, Di Mattei a macaco.

It is singular that, while positive results of the injection of mala-

rial blood from man to man are almost constant, contradictory results have been noted in experimental attempts to transmit the disease from one bird to another, even of the same species and variety. Celli and Sanfelice claim to have seen the disease transmitted in this way in certain cases, but Grassi and Feletti and also Di Mattei have always had negative results.*

Passage of Malarial Germs through the Placenta.

Whether such a condition as congenital malaria exists is a question still discussed, and even answered affirmatively by some writers, although in our opinion there have never yet been reported any well-studied cases which demonstrated in a way to silence all objection the possibility of the infection of a fœtus in the mother's womb. Most observers, although having at their disposal a great amount of material for study, have never seen a child with malarial fever immediately after birth, nor have they succeeded in finding the parasites in a fœtus removed from the womb of a woman dead of pernicious fever in whose blood was an enormous number of germs, or in one born as a result of abortion occurring shortly before the mother's death. Thus Bignami found no parasites in the blood of two fœtuses, one of three, the other of six months, coming from women gravely ill of malaria, and a similar negative result was noted in a third case occurring in the San Giovanni Hospital. In a pregnant woman dead of pernicious fever, after numerous previous malarial attacks, the parasitoscopic findings common in pernicious fevers were noted at the autopsy, and the liver and spleen presented the changes usually observed in malarial cachexia; but there were neither parasites nor malarial lesions in the fœtus. A negative observation similar to those of Bignami has been published by G. Bastianelli, two by Caccini, and one more recently by Thayer. Many observations of a like character have been made in Rome but never published.

These facts, while naturally they do not exclude the possibility of the passage of malarial parasites through the placenta, nevertheless demonstrate that even if such a thing as a congenital malarial infection exists it is at least exceedingly rare and exceptional. In order to explain this absence of malarial parasites in the blood of the fœtus (which is certainly the rule) Bignami invokes the fact that the malarial parasites show no tendency whatever to wander out of the blood-vessels. Even in the case of small capillary hemorrhages, which are

* From a recent paper by R. Koch (Zeitschrift für Hygiene, xxxii., 1899), it appears that according to the researches of Pfeiffer the halteridium is not transmitted from bird to bird by means of infected blood, whereas the proteosoma is thus conveyed with great facility. This may explain the apparent contradiction.

sometimes very numerous in the brain in comatose pernicious fever, no parasites are found in the extravasated red blood corpuscles, although they are present in great numbers in the blood within the vessels of the neighborhood; the red corpuscles in the bloody exudate in malarial pneumonia also contain no parasites. All these facts make it appear very improbable that the parasites can pass from the maternal to the fœtal circulation during their intraglobular existence. If, however, it were held that this passage is possible during the brief period in which the very young parasites are living free in the blood plasma, in order to explain the negative results above mentioned we should have to assume that the fœtal blood is not adapted to the development of the parasites. In support of this hypothesis we might adduce the fact noted by Bignami and Guarnieri that the plasmodia do not invade the nucleated red corpuscles and most probably not the young red globules.

In opposition to the observations above cited, in which the results of examination were negative, we find others, very few, however, which according to some authors would furnish an absolute demonstration of the passage of malarial germs from the mother to the fœtus. Thus Laveran cites two observations, one of Bouzian and the other of Bein, which, he asserts, prove beyond doubt the existence of congenital malaria. We have been unable to find the original report of the first of these cases, but Bein's case is, in our opinion, not above criticism since the presence of malarial germs in the child's blood was noted too long a time after birth and the possibility of post-natal infection could not be definitely excluded. Mannaberg cites as positively conclusive a case published by Ducheck in 1858. The case was one of a child whose mother had suffered from intermittent fever during her pregnancy, and who died three hours after birth; at the autopsy there was found a notable enlargement of the liver and spleen together with much black pigment in the form of large irregular lumps and granules in the spleen and in the blood of the portal vein. Without entering into a discussion of this case, the original report of which we have been unable to procure, we would call special attention to the fact that no truly demonstrative case of congenital infection has been published since the discovery of the malarial parasites. Evidently we cannot accept unquestioningly those observations in which the authors felt themselves justified in making a diagnosis of congenital malaria from the simple finding of an enlarged spleen which might have been due to some other cause, for example, syphilis, or from the existence of intermittent febrile paroxysms in a new-born child.

While on this question of so-called congenital malaria, we would

report an observation recently made by Bignami, who has demonstrated that in the new-born children of malarial mothers we not only do not find the malarial parasites, but not even an anæmic state such as we might naturally expect to find, given the anæmic or even cachectic condition of the mother. It is really surprising to find the fœtus from a dead woman, who had suffered from marked malarial anæmia and who was suffering from an acute malarial attack at the time of death, full blooded and without any of these parenchymatous alterations of the viscera, for example, the liver, present in so marked a form in the mother. A fact such as this leads us to believe that the fœtus enjoys a certain immunity even against the secondary effects of infection in the blood and parenchyma of the viscera.

Incubation.

As in other infectious diseases, so also in malaria, there is a period of incubation from the moment of entrance of the parasites into the organism to that of the development of the disease. Concerning the duration of incubation in spontaneous malarial infection many writers hold that it may vary from a few hours to many weeks or even months. According to Hertz the duration of the period of incubation usually varies between six and twenty days. The belief that the infection may declare itself a few hours after the entrance of the pathogenic agent is quite widely diffused among physicians in general and is held even by well-known authorities, such as Salisbury and Hertz. But the proofs alleged in support of this belief will not stand against a somewhat severe criticism. For example, we cannot accept as evidences of malaria developed immediately after exposure to the noxious agent the malaise accompanied by smarting and dryness of the throat, nausea, etc., experienced by Hertz and Salisbury a few hours after they had been exposed to the emanations from marshes in the process of being dried up. We have never seen a case of malaria after such a brief incubation period. Furthermore, from what we know of the life cycle of the parasites of the intermittent fevers and of the mode of infection, we can affirm that the reported cases of fever without incubation or with an incubation of a few hours only were not of a malarial nature, and that such an occurrence, even though we may not exclude its theoretical possibility, can practically be regarded as non-existent.

In order to determine exactly the duration of the period of incubation in spontaneous malaria, it is necessary to take account chiefly of those cases in which the time and duration of the stay in a malarious regions can be ascertained with certainty. We have had the op-

portunity of observing some cases of this kind, of which we will give here a brief summary.

Case I.—A robust young man, 25 years old, living in the central part of Rome, had never had malarial fever. On November 4th, 1894, he was obliged to go to Sermonetta, a notoriously malarious town near the Pontine marshes. He arrived in the city at ten o'clock, slept that night, tormented with swarms of mosquitos, in a house on the outskirts of Sermonetta, and in the morning returned to Rome, where he resumed his usual occupation. For six days he was in good health; then he had two days of malaise, and on November 13th, that is to say, *nine days* after his stay in Sermonetta, he was taken down with an estivoautumnal tertian fever, the parasites of this form being found in the blood.

Case II.—An engineer living in Rome, who had never suffered from malaria, was constrained by the duties of his calling to pass a day in October, 1895, in a place in the Pontine marshes, and he slept that night in a cabin in poor repair in which were many mosquitos. At the end of *ten days* an estivoautumnal infection with irregular fever developed, of which he had several relapses extending up to the following spring.

Case III.—A lady, who for many years had enjoyed good health, passed a week at Fiumicino in the month of October, 1894. Three days after her return to Rome an estivoautumnal malarial infection declared itself, and, not being promptly treated, developed into a choleraic pernicious attack; following this the patient remained profoundly anæmic with a sanguinolent diarrhœa, and died at the end of a few weeks.

In the last case, assuming that the patient had been inoculated with the pathogenic germs on the first night after her arrival at Fiumicino, the period of incubation could not have exceeded *ten days*. Of the three cases, therefore, the duration of the incubative period was in the first nine days, in the second ten, and in the third not more than ten. When the sojourn in the malarious district has been very long, it is of course impossible to estimate with any approach to certainty the duration of the incubation.

From the histories of patients received into the hospitals of Rome, we may conclude that when one stays in a malarious region during the fever season, for example, in the Campagna in the autumn, the infection manifests itself after a period ranging from eight or ten days to a month. Furthermore, from reports gathered in malarious regions we learn that the period of incubation grows shorter with the advance of the fever season. This fact is in accord with the others of the increased number of mosquitos which are found to be infected in the late autumn; and we can readily understand that when inoculation with a large quantity of sporo-

zoites is thus facilitated, the infection will be likely to declare itself more rapidly.

Certain writers have reported cases in which there was an incubation period of several months. Thus Bloxall (quoted by Hertz and Sternberg) cites the following case: A war-ship remained three days at Port Louis; after its departure four sailors fell ill, two of quotidian fever after twelve and fourteen days respectively, and two of tertian fever, one after forty-eight, the other after one hundred and eighty-four days. To these cases of very long incubation may be compared those in which the infection, after the first febrile paroxysms, remains latent for a long time, but is not extinguished, as is shown by late and sometimes exceedingly late relapses.

How can be explained these instances of incubation of long duration? Do the parasites collect in the spleen or in the bone-marrow and there remain inert until stirred into activity by some one of the exciting causes? Or do the parasites multiply indeed, but in such small numbers as to occasion simply a non-febrile infection which gets well spontaneously; so that the first and late febrile manifestation is really a relapse? This last is the hypothesis advanced by Thayer and accepted by Mannaberg. In corroboration of it might be adduced the fact, now verified by many, of the succession of parasitic cycles without any febrile manifestations, and the further fact of anæmia and splenic enlargement in persons living in malarious regions who deny ever having had any attacks of fever.

We have recorded cases of incubation of spontaneous estivoautumnal infection, but we have had no opportunity of studying it in cases of simple tertian or quartan fever. There are, however, reasons for believing that the period of incubation in these varieties of malarial infection is of longer duration, and longer in quartan than in tertian, one cause for such belief being the different duration of the life cycle of the quartan and of the tertian parasites.

It is said that the period of incubation varies according to the period of the malarial season, but it must also vary according to the individual conditions, and according as other occasional causes, such as a chilling of the body, digestive disturbances, excessive labor, etc., enter into activity or not. The influence of the first of these accidental causes is shown in the development of an estivoautumnal endemic among many laborers after a sudden fall of temperature, a rain storm, etc. In these cases there can be no doubt that the infection had already occurred some time before and that it was aroused from its condition of latency into activity because the circulatory disturbance, and perhaps also some other functional changes following a chilling of the body after great heat, either weakened the or-

ganic resistance against the pyretogenous action of the parasites or increased the multiplicative activity of the latter so that the quantity necessary to cause fever was soon reached.

The study of the incubation period leads to more positive results when the infection is artificially produced by intravenous or subcutaneous injection of malarial blood. It may, however, be assumed *a priori* that the duration of the period of incubation in cases of experimental infection does not correspond to that of spontaneous infection, for in the latter there is an inoculation of sporozoites developed in the body of the mosquito, while in experimental infection there is an introduction of parasites already habituated to an existence in human blood. The results of experiments of this nature are set forth in the following table compiled from those of Bignami and Bastianelli and of Mannaberg. We find first the cases of inoculation with quartan blood, then those with blood containing the parasites of simple tertian, and finally those with blood charged with estivo-autumnal parasites. In this table no account is taken of Gerhardt's cases, because they are not accompanied with a report of the parasites present, nor of two cases of Gualdi and Antolisei, because in them the infection was a mixed quartan and estivoautumnal, nor of any cases with negative results, nor finally of those of Celli and Santori, because in them attempts at immunization with blood serum of immune animals preceded the inoculation with malarial blood.

TABLE OF EXPERIMENTAL INOCULATIONS WITH MALARIAL BLOOD, SHOWING THE DURATION OF THE INCUBATION PERIODS.

Number.	Author.	Parasites in the inoculated blood, and febrile type.	Quantity of blood inoculated and of parasites contained therein, and mode of inoculations.	Days of incubation.	Febrile type, and parasites reproduced.
1	Gualdi and Antolisei.	Sporulations. Quartan.	2 c.c. Many parasites. Intravenous injection.	15	Quartan. Quartan parasites.
2	do.	do.	(?) do.	12	do.
3	Baccelli.....	do.	4 c.c. A moderate number of parasites. Intravenous injection.	12	—— Quartan parasites.
4	Di Mattei	Parasites. Quartan..	5 c.c. Subcutaneous injection.	18	Quartan. Quartan parasites.
5	Calandruccio ...	do.	1 c c. do.	18	do.
6	Di Mattei	do.	2 c.c. do.	11	do.
7	Antolisei and Angelini.	Tertian parasites with and without pigment, at the beginning of the fever.	(?) Intravenous injection.	11	Double tertian. Tertian parasites.
8	do.	do.	1½ c.c. do. ..	11	Irregular fever, then tertian. Tertian parasites.
9	Bein	Tertian parasites.....	2 c.c. Blood taken from patient by means of leeches.	12	—— Tertian parasites.
10	do.	do.	2 c.c. do.	12	Double tertian. Tertian parasites.
11	do.	do.	2 c.c. do.	9	Tertian. Tertian parasites.
12	do.	do.	2 c.c. do.	9	Tertian, first simple, then double. Tertian parasites.

TABLE OF EXPERIMENTAL INOCULATIONS WITH MALARIAL BLOOD, SHOWING THE DURATION OF THE INCUBATION PERIODS.—*Continued.*

Number.	Author.	Parasites in the inoculated blood, and febrile type.	Quantity of blood inoculated and of parasites contained therein, and mode of inoculations.	Days of incubation.	Febrile type, and parasites reproduced.
13	Baccelli.....	Tertian parasites taken during the chill.	3 c.c. Intravenous injection.	6	Tertian. Tertian parasites.
14	Mannaberg.....	Tertian parasites.....	$\frac{1}{2}$ c.c. of the centrifugated corpuscular sediment, subcutaneously.	21	do.
15	Bastianelli and Bignami.	Estivoautumnal parasites, plasmodia with granules.	2 c.c. Blood rich in parasites.	2	Irregular fever. Estivoautumnal parasites.
16	do.	do.	5 c.c. Moderate number of parasites.	2	do.
17	do.	do.	$\frac{3}{4}$ c.c. do.	5	Estival tertian. Estivoautumnal parasites.
18	do.	do.	$\frac{1}{8}$ c.c. ——	4	Irregular fever. Estivoautumnal parasites.
19	Bignami.....	Estiovautumnal parasites. Malignant tertian.	Subcutaneous injection of less than one drop of blood.	6	Malignant tertian. Estivoautumnal parasites.
20	do.	do.	do.	10	do.
21	Di Mattei.....	Estivoautumnal parasites, small amœbæ and crescents.	2 c.c. Subcutaneous injection.	14	Irregular fever. Estivoautumnal parasites.

From their own table Bignami and Bastianelli derive the following averages for the period of incubation in the different types of fever: Quartan—maximum 15 days, minimum 11, mean 13; simple tertian—maximum 12 days, minimum 6, mean 10; estivoautumnal fevers—maximum 5 days, minimum 2, mean 3.

Mannaberg, in his latest book, published in 1899, adding other experimental cases, records the following incubation periods: Five cases of quartan—11 to 18 days, mean 13.4; seven cases of simple tertian—6 to 21 days, mean 11; seven cases of estivoautumnal fevers—3 to 14 days, mean 6.5; two cases with blood containing crescents, but without (?) amœboid parasites—13 to 15 days, mean 14. Concerning these last two cases, Mannaberg adds that in addition to the crescents there were "probably" also amœboids in the inoculated blood; we should say that these last-named forms could "positively" have been found, though possibly in very small number, in the inoculated blood, for we now know well that the crescent forms do not multiply in human blood and are not pyretogenous.

From the cases collected by ourselves we derive the following: for quartan, the maximum incubation period is 18 days, minimum 11, mean 14.3; for tertian, maximum 21 days, minimum 6, mean 11.3; for summer-autumn fevers, maximum 14 days, minimum 2, mean 6.1. These figures are almost identical with those of Mannaberg given above. We may add that from some quite recent experiments of Celli, it appears that the incubation period of experimental quartan may be very long, extending even to more than two months.

Comparing the results of all the inoculations made up to the present, it is shown very clearly that the minimum and the mean lengths of the period of incubation vary according to the variety of infection, being longer in the quartan than in the simple tertian, and in the latter than in the estivoautumnal. The varying duration of incubation in the different forms of fever is evidently dependent upon the varying duration of the life cycle of the corresponding parasitic forms as well as upon their varying capacity for multiplication, as Bignami and Bastianelli have already noted. Taking into account this latter property we readily understand why the incubation period of the estivoautumnal tertian is shorter than that of the simple tertian.

In addition to the properties inherent in the various species of parasites, the duration of the incubation period is also determined by the quantity of blood, that is to say, of parasites, infected. Thus, while with an injection of 1.5 c.c. of blood containing tertian parasites at the beginning of the fever there was an incubation period of eleven days, with one of 3 c.c. of blood containing the same parasites and taken during the same stage of the febrile attack the period of incubation lasted only six days. These differences are readily explained by the evident fact that the greater the number of parasites injected the more speedily will a number sufficient to cause fever be attained by the process of multiplication.

But a new approach to a determination of the exact duration of the period of incubation is opened up by the recent discovery that malaria in man is the result of inoculation by infected mosquitos. It will be readily seen, however, that we must not draw our conclusions from cases in which persons in a non-malarious region have slept for an indefinite period in a room containing infected mosquitos, but only from those in which the subject of the experiment was stung by the mosquitos at a known moment and then at once removed from the possibility of being stung again.

Up to the present writing there have been, so far as is known, but two experiments of this sort made with infected individuals of Anopheles claviger. In the first of these A. F—— was stung by two mosquitos on December 10th, by two on the following day, and by four on December 13th. The first febrile paroxysm occurred December 29th, and following this there developed an ordinary double tertian; the period of incubation was therefore between sixteen and nineteen days. In the second experiment A. B—— was stung on January 2d by three specimens of Anopheles which had sucked blood containing crescents and had then been kept for several days in an incubator at 30° C. (86° F.). On January 5th new punctures were made by two of these same three mosquitos. The fever, an estival ter-

tian, declared itself on January 14th; the period of incubation was therefore from nine to twelve days.

In these experiments of inoculation by infected mosquitos there was reported precisely what happens when one acquires malaria in the ordinary manner, for there can be no difference between the sting of an infected mosquito received by a man asleep in a region where malaria prevails, and one of the same sort of mosquito received by a man in a salubrious region while the mouth of a test-tube containing the infected insect is applied to the subject's skin.

We have in these two experiments a confirmation of the longer duration of incubation in simple tertian than in estival tertian. We also note that the duration of the period of incubation in this estival tertian, produced experimentally by inoculation with infected mosquitos, corresponds to that observed in the cases above mentioned of estivoautumnal infection contracted during a brief sojourn in a malarious region.

Occasional and Predisposing Causes.

Now that we know with certainty the true cause of malarial infection we can assign the just value to the so-called predisposing and occasional causes. We can readily understand that these should not be spoken of as true causes since they could not, even all acting together, produce malaria without the presence of the pathogenic agent in the organism; they are simply conditions which favor inoculation with the parasite and the development of the latter within the body. To these secondary causes, especially to some of them, many physicians formerly attached the greatest importance, and so do even yet those who have not kept up with the most recent discoveries concerning the etiology of malaria, as we shall see in the following rapid sketch.

Racial predisposition has been much discussed, but it would appear that most of the individuals in every race are liable to infection, the only qualification of this statement being that those living in malarious regions acquire a greater or less power of resistance to the development of the infectious agent. Perhaps other conditions being equal the negro suffers less frequently, especially from the pernicious forms of malaria. This view is strengthened by the recent studies of Thayer and Hewetson on the malarial fevers of Baltimore.

Malaria spares no *age*, but infants and children are more subject than adults to infection. This is explained, at least in part, by the predilection of mosquitos to attack delicate skins and by the profound sleep of the young.

There is no difference between the *sexes* as regards predisposition

to malaria, men and women when equally exposed suffering equally. But there is a difference as regards exposure among the inhabitants of healthy cities surrounded by a malarious country, work in which is performed chiefly by the men. In such cases the women are for the most part exempt, as we see in certain cities lying above the Pontine marshes where the women, who stay at home and attend to the household affairs, are usually healthy and vigorous, and many of them several times widowed, while the men suffer from repeated attacks of malarial fever, become cachectic, and die early.

The influence of *occupation* is evident in places in the vicinity of malarious centres. While those whose work takes them into the insalubrious country are prone to acquire malaria, laborers who are busied in the cities are exempt, with the exception of those who live in houses on the outskirts of the city overlooking the open country. But even among the field laborers we must distinguish between those who remain in the country only during the daytime and those who also pass the nights there, the latter being more subject to malarial attacks; also between those who work in the winter and spring, who do not take the disease or only in a mild form, and those who labor in the fields during the summer and autumn, who suffer more frequently and often from the pernicious fevers. Thus, in the Roman Campagna, the laborers who harvest the hay in the spring are free from infection or at most suffer only from a simple tertian fever, while those who harvest the grain, and especially those who thrash it and engage in other of the autumnal works, pay a heavy tribute to malaria. Besides agricultural laborers, those are also subject to malaria who are obliged to work at ditching, excavating, and other tasks, such as railroad building, the construction of fortifications, the diking of rivers, etc., which necessitate an upturning of the soil. Brick-makers who work at kilns in malarious regions, soldiers, carters, hunters, and others who pass both days and nights in unhealthy districts are also subject to the infection.

To these individual conditions of infection are to be added others which exercise a certain and powerful influence upon the development of the primary infection as well as of relapses.

The greatest importance is attached to a *chilling of the body*. Indeed, this was formerly regarded as the true cause of malarial fever by those who, rejecting the theory of a miasm, explained the evil influence of marshy regions chiefly by the difference between the diurnal and the nocturnal temperature. But the importance of a chill as an occasional cause of infection is derived from clinical and endemiological observations. Sufferers from fever frequently give a history of a cold by which they were attacked some time before the

onset of the fever; relapses often declare themselves after a cold bath or a cold douche; and both primary infections and relapses not rarely attack those who ascend from the warm plains to the cool mountain regions. Furthermore, the beginning of the malarial season is announced when, after a few days of great heat followed by rain and a sudden cooling of the atmosphere, several men fall ill almost simultaneously. It is after from one to three days of a fall in temperature during July that sufferers from malaria begin to enter the hospitals in cities surrounded by malarious plains, and the first cases of the season of pernicious fever are encountered.

Much importance was formerly, and still is popularly, attributed to imprudences in diet, and residents in malarious countries believe that the fever may be caused by the eating of much fruit, especially unripe fruit. Although this is not strictly true, we can nevertheless readily understand how the organism may be rendered less resistant to the action of the parasite if any imprudence in diet is followed by gastroenteric disturbances, especially by a weakening diarrhœa. Perhaps also the localization of the parasites in the capillaries of the gastroenteric mucosa, as occurs in choleraic pernicious fever, may occur more readily when circulatory changes excited by digestive disturbances already exist.

Excessive labor, especially in the sun, mental disturbances, and insufficient food are all factors in the weakening of the organism which renders it more susceptible to the development of the specific infection. When we consider the nature of the food of the poor field hands in malarious regions, how they are clothed, how and where they sleep, in what way and how much they labor, we readily perceive that all the factors which facilitate the entrance of the malarial germ and its development within the organism are there existent.

Immunity.

We have seen the mechanism of malarial infection in man and what are the occasional and predisposing causes favoring the process in individual cases, but we shall now see that, even when placed under the same conditions of surroundings and of life, not all races nor all individuals exhibit the same proneness to acquire malaria.

Although there are no races originally immune to malaria, we are forced to recognize the fact that the various races present a varying degree of resistance to the infection. Thus negroes inhabiting malarious regions in the tropics are less subject than white men in the same places to the grave forms of malaria, and having once been infected they acquire, so it is asserted, a relative immunity more readily

than the whites. But it must be said that authors are at variance concerning this question of racial immunity. Some attribute to the blacks an almost complete immunity, but this, as recent observations have demonstrated, is an error; possibly the various races of negroes differ among themselves in this regard. The inhabitants of the Kamerun coast, according to F. Plehn, seldom have fever, and when they do the febrile paroxysms never last more than a few hours; they rarely ask the Europeans for quinine and usually recover spontaneously in a few days. But a change of residence deprives them of this relative immunity, at least temporarily. Hæmoglobinuria, which prevails in very grave form among the Europeans in Kamerun and in other places, has been noted also among the natives, but F. Plehn did not see a single case among two hundred and seventy-six cases of fever observed by him in the negroes.

According to L. Martin, whose observations were made in Sumatra, the Malays and the Javanese enjoy a certain degree of immunity, suffering for the most part from mild forms only of the disease, and the Tamils, although they are chiefly occupied in hard field labor, are still less predisposed to malaria.

But even in the same race there is often observed a varying power of resistance among the inhabitants of different regions. Thus we know that in Italy the peasants of Venetia and of the Marches suffer terribly from malaria when they come to work in the Roman Campagna, while the inhabitants of Abbruzzo and of the mountainous parts of Latium possess in general a greater power of resistance. Tommasi-Crudeli attributes this resisting-power to a natural selection effected by malaria upon the population, the inhabitants of these regions having for centuries had the custom of descending from their native mountains into the insalubrious plains during the season of agricultural labor.

But apart from that we find among the fixed population of malarious regions evidences of great variability in individual resistance to infection and even examples of veritable immunity. We have to distinguish a congenital immunity, which may be a family peculiarity, and an acquired immunity, which in the great majority of cases is not complete but only relative. Before asserting the complete immunity of any given individual, however, we must be sure that he has lived a sufficiently long time in a place where grave malaria prevails. Indeed, we may sometimes see individuals, who have been for a year or even longer in malarious regions without contracting the disease, suddenly fall victims to a grave infection when they had come to regard themselves as perfectly secure. It is necessary also to be certain that the person in question has actually been inoc-

ulated with the malarial germ. We know indeed that some, who have always carefully avoided sleeping out of doors or with open windows, who have always slept under a mosquito bar, who, in a word, perhaps unconsciously have always so conducted themselves as to avoid being stung by malarial mosquitos, have for a long time escaped infection. In such cases, of course, we have no right to speak of a peculiar resistance to infection or of immunity.

Examples of *congenital immunity* in our race are exceedingly rare, yet they are to be found in all malarious districts upon diligent inquiry. Whoever has frequented places where grave malaria exists has known of some such cases; he has seen robust persons of good color who tell him that they have never had the fever and who have no enlarged spleen. A physician of Sermoneta, a region of savage malaria, in which the fever rages as it does in the Campagna, the women and children habitually suffering from it, has told us that there are there robust old men who have lived for more than seventy years without ever having had the fever. But these are exceptional cases, and the village has been nearly depopulated in the past fifty years.

This immunity may be transmitted to the descendants. An example of *family immunity* has been noted by us in a gravely malarious settlement near Nettuno. It was in the person of a robust man about forty-five years old, an overseer of a number of agricultural laborers, who was born in a malarious region, but had never suffered from malaria. He said that his father, born in a salubrious place, had come to the Campagna, but had never had a fever, and died there at an advanced age. His mother suffered from malaria for many months at the beginning of her residence in a malarious district near the Pontine marshes, then she recovered, and was never after troubled. The sons of this man are also immune; one of them is a lad of sixteen years, tall, well developed, and of a good color; another is a boy between nine and ten years, who is also in excellent health; yet both of them live between Conca and Campo Morto, places of ill repute even in the Roman Campagna on account of the virulence of the malaria prevailing there. With this family, exceptional in its immunity, contrast strangely most of those living permanently in the same place; among these the adults usually succeed in moderating the effects of the disease by means of specific treatment, but the children generally suffer irreparable injury.

Examples of *acquired immunity* are less rare. We sometimes see individuals who suffered from malaria, during the first year of their sojourn in the infected region, for many months—usually from the summer or the autumn to the spring of the following year, but after that remained well—having a fairly healthy appearance and being

capable of considerable work, but on examination they are found to be suffering from enlargement of the spleen often of considerable size. There are also persons who, during a residence of many years, even fifteen or twenty, in a markedly malarious region, have never suffered from attacks of typical malarial fever, but are troubled from time to time with a slight feverishness which they attribute to an imprudence of some sort, but which is probably a very mild malarial attack excited by overwork or exposure. These individuals look fairly well and they preserve their strength and ability to work often to an advanced age, but examination shows the presence of an enlarged spleen. In the first of these cases there is a more or less complete acquired immunity following a series of febrile attacks; in the second the individual is endowed from the first with a marked power of resistance in consequence of which the infection has never run an acute or grave course, such as it usually does in new arrivals in malarious places, but has rather been chronic from the beginning, and during this time the subject has been gradually increasing his initial resisting-powers until he finally acquires quite a notable degree of immunity. It should be stated, however, that we have seen examples of this sort among the permanent inhabitants of malarious regions only in the class of overseers, stewards, and agents, those, namely, who are well fed, relatively well housed, and are not obliged to labor, but usually pass the day on horseback directing the laborers and superintending the work. We have never seen a case of acquired immunity in an ordinary laborer, and evidently this sort of immunity can be developed and maintained only under the most favorable conditions.

Here we ought to say that the word "immunity" is employed by some writers to designate only an absolute resistance to infection, that is to say, in the sense that the parasites do not develop after inoculation; while for those cases in which the infection is actually acquired and persists, as evidenced by the relative anæmia and enlargement of the spleen, but in which febrile reaction is absent or slight and the pernicious fevers never occur, they reserve the term, "relative tolerance" (Kelsch and Kiener and others). We use the term immunity also to express this limited power of resistance of the organism, but we qualify it as incomplete or partial.

Now, while in the rare instances above referred to there is established a relative immunity of great practical value, in the vast majority of cases the organism acquires gradually a certain degree of resistance to the infective agent, but this resisting-power is not sufficiently strong to prevent relapses from time to time which finally induce a cachectic condition. Although, at first thought, the application of the term acquired immunity to cases of this sort, in which

the individual is reduced to such a wretched state, does not seem justifiable, yet we find many proofs that such subjects are really more resistant to the action of the malarial germ than new arrivals who have never had the fever before. It is seen, for example, that pernicious attacks are almost always primary or at least occur with the first relapses; it is common to find the spleen very soft and but slightly enlarged at the autopsy of one who has died of a pernicious fever, and on the other hand it is rare to find the characteristic parasitic condition of pernicious infection in an individual with a pronounced chronic enlargement of the spleen. It is perhaps correct to say that the majority of those who become cachectic after a long sojourn in a malarious region do not die of malaria, but of its consequences, and generally of complications such as pneumonia and the like. At the autopsy of such a cachectic subject who has remained up to the end of his life in a malarious region, the enlarged spleen is usually found of a bright red color on section and without trace of melanosis, excluding, therefore, a recent malarial infection.

But the proof that a relative immunity is gradually established during malarial infection is furnished by a study of the ordinary course of malaria both in those who leave the district after having acquired the fever and in those who, remaining there, are continually subject to reinfection. In most cases the ordinary series of events is as follows: A laborer, going to work in the Campagna during the month of July, usually takes an estivoautumnal fever which begins with severe and prolonged attacks and sometimes with a true continued fever; cured of this attack with quinine, he has regularly a relapse after one or two weeks, but in the relapse the paroxysms are more distinctly intermittent and less protracted. Other relapses follow this one at longer and longer intervals up to about one month, rarely longer, these relapses being represented by groups of attacks of diminishing intensity and finally by isolated paroxysms with slight elevation of temperature. By the following spring, as a general rule, the infection will have gradually spent itself.

The same thing is observed in tertian or quartan fever, except that in the tertian fever, as we know, if the treatment is instituted properly and at the beginning of the disease, we usually succeed in reducing to a minimum or even averting entirely the relapses; while in quartan fever the groups of attacks follow each other with the greatest obstinacy, separated by longer or shorter intervals of apyrexia —a fact well known to physicians from the most remote period—and the infection in some cases dies out only after many years of existence, even when the patient is living under the most favorable conditions.

It might be supposed that this course was purely the effect of the specific medication. Indeed, most patients succeed in overcoming the fever, after a certain number of attacks, with quinine; then after a certain period of apyrexia the relapse occurs, the mildness of which, compared with the primary attack, might be thought to be due solely to the fact that the infection has been attenuated by means of the remedy. We may, however, affirm with certainty that this course is owing not alone to the treatment adopted, but in great part to the modifications occurring in the human organism during the existence of the infection. Indeed, if we refrain from giving quinine to patients not suffering from a grave form of the disease, we find that, after a certain number of febrile paroxysms, the upward temperature curves tend to become less marked and a spontaneous recovery takes place. But this cure is temporary only, and after a variable interval of time the fever returns, usually in milder form. And not only is the fever less pronounced, but all the effects upon the organism of the malarial poison manifest themselves in the successive relapses in continuously lessening degree. Thus we find that patients, under equal conditions as regards the quantity of infection, become less anæmic in the relapses than in the primary attack. For a fuller discussion of this point the reader is referred to the section on pathology. It was evidently in this way, by passing through a series of relapses, each one milder than the preceding and recurring at progressively longer intervals, that the subjects of malaria were cured before the virtues of cinchona bark were discovered.

The way in which this spontaneous cure is effected is still a matter for discussion (see the section on treatment), but there can be no doubt that it is primarily connected with the modifications which the infection itself produces in the human organism. Indeed, we cannot explain it at all unless we admit either a progressive attenuation of the parasites until they have gradually lost their pathogenic action and their capacity for multiplication, or a progressive increase in the patient's power of resistance to the pathogenic action of the parasites—in other words, an acquired immunity. While not denying absolutely that the pathogenic action of the parasites may be weakened in the course of time, we yet cannot attach very great importance to their attenuation. Indeed, as we have already noted, patients with chronic malaria, even when they remain permanently in a malarious region, where they are continually subject to reinfection with fresh virulent material, do not, as a rule, suffer from the grave forms of malaria as do the new arrivals. Furthermore, experiments have demonstrated that, when blood containing very few parasites taken from a person who has spontaneously recovered from an attack of malaria is injected

into a healthy person, a grave form of infection may be induced in the latter. It is therefore the organism of the patient himself which prevents the parasites from developing their pathogenic action, and not the parasites which have lost of themselves their power of exciting disease. Then again, when a patient, say one suffering from a quartan fever, in whose blood the parasites regularly pass through their life cycle without inducing pyrexia, is seized anew with febrile paroxysms, in consequence of the action of some occasional cause, such as a cold bath, for example (see the discussion of the febrile paroxysm in the section of Pathology), are we not forced to the conclusion that this is due to the fact that the defensive capacity of the organism has been weakened by the action of cold?

Everything, therefore, leads us to the conclusion that certain changes take place in the patient during the febrile attacks through which he acquires an immunity from the effects of the microorganism. But this immunity, as is the case also in certain other infectious diseases, is of brief duration, and when it is weakened the relapse occurs; this confers anew upon the patient an increase of his power of resistance whence again a cessation of the attack and a new period of apyrexia. After every apyretic period a part of the acquired immunity persists, and this is the cause of the diminishing intensity of the successive attacks and of the fact that even reinfections do not, as a rule, take on a grave course.

In this way, it appears to us, may be explained satisfactorily the usual course of the malarial infection. The results of the researches made up to the present time do not permit us to analyze this acquired immunity of malarial subjects in the same manner as has been done in the case of some other infectious diseases. Attempts to confer immunity upon man artificially have hitherto failed (see the section on Treatment), but we must remember that the experimental study of this question in the human subject is attended with great difficulties. We have reason, however, to hope that, as has happened in the case of other questions concerning the biology of the hæmosporidia, this problem also, which is of such great importance, may have new light thrown upon it through the study of analogous affections caused by hæmatozoa in animals and especially in the mammifera.

What now is the practical value of this relative immunity acquired by malarial subjects after a long course of the infection? Evidently in the great majority of cases it is very small; for if we except the small number of persons who become immune after a few months of fever to such a degree that they can remain in the malarious region without suffering from further attacks, most subjects acquire this relative immunity at the cost of a chronic infection or of cachexia.

This causes a progressive degeneration of the races living in regions of intense malarial prevalence, and hinders that natural selection through the action of which we might *a priori* look for the creation of an immune race. Very long experience has demonstrated the fact that not only does this selection in the case of the white races not act in such a way as to produce practically useful results, but that the agricultural population of malarious regions is constantly being thinned out and must be continually recruited by laborers coming from non-malarious districts.

Whether there are other diseases which confer a certain degree of immunity from malaria we are unable to say. There is a very widely entertained belief that some skin diseases afford protection against the fever. This is asserted by many in the tropics, especially in the case of lichen tropicus and tropical furunculosis, infections due, it is believed, to the staphylococcus pyogenes aureus (F. Plehn). The same opinion, according to Van Der Burg (quoted by F. Plehn), is held in India. But, admitting the correctness of the observations, we may yet ask whether we have to do here with a true immunity conferred by the cutaneous affection or whether these patients escape malaria because, owing to the condition of their skin, inoculation of the malarial germs by mosquitos is impossible. A fact which tends to render the latter the more probable is that, so it is asserted, those subjects are exempt from malaria only so long as the cutaneous affection lasts. We may therefore assume that, if the infected mosquitos do puncture the inflamed skin, the malarial sporozoites do not find in the blood-vessels of the infiltrated territory the conditions essential to their development.

The Malarial Environment.

Before bringing to a close the study of the etiology of malaria, we must speak of the malarial environment, or, as Celli expresses it, the localistic predisposing causes. Although epidemics and pandemics of malaria have been described, the affection usually prevails endemically in certain localities, and in some it has prevailed for centuries.

Geographical Distribution.

Malaria is one of the most widely diffused of all diseases in warm and temperate regions; most prevalent and intense near the equator, its extent and gravity decline in proportion to the distance north and south from this line. In many parts of the world malaria has died out, or occurs only in its milder forms.

In *Europe* malaria prevails in the peninsulas and islands of the Mediterranean, and also exists in the central parts. In Russia the disease extends from the steppes on the shores of the Caspian Sea, through the valley of the Volga, on the steppes of the Caucasus, and along the northern coast of the Black Sea, and it follows the Dnieper and the Dniester, and up the valley of the Danube. In Germany a mild form of malaria is found in the plains of the north, especially in certain parts of the course of the Vistula, Oder, Elbe, and Weser. In Holland and Belgium, where formerly pernicious fevers were very common, malaria is now greatly reduced in extent and virulence owing to the intelligent and constant labors which have resulted in the construction of the celebrated "polders." In France malaria of a mild type is found along the west coast. On the Mediterranean shore the region of malaria extends from Perpignan to the delta of the Rhone, and inland to the valley of the Saône. In Austria-Hungary malaria follows chiefly the course of the Danube and its confluents, extending also down along the coast of Istria and Dalmatia. In the Balkan peninsula many and dangerous centres of malaria are found in the valleys and at the mouths of the rivers. Greece is gravely afflicted with malaria on most of its coast line, and, of the islands, Crete, Cephalonia, Zante, Corfu, and Santa Maura are malarious.

In Italy and the islands belonging to it malaria is severe and widespread. In the north of Italy districts where malaria prevails in mild form are found in Piedmont, especially in the province of Novara, and in Lombardy, especially in the province of Cremona. In Venetia and Emilia malaria exists in the provinces of Venice, Padua, and Rovigo, and in that of Ferrara from the mouth of the Po to the Valle di Comachio. But it is in central and southern Italy that we find the most extensive areas of grave malaria. Central Tuscany, Umbria, and the Marches contain circumscribed areas of generally mild malaria. The infection begins on the shores of Tuscany from Viareggio and assumes considerable intensity descending towards Grosseto, where it becomes very grave; thence it follows along the coast of Latium where are found the notorious malarious regions of the Roman Campagna and the Pontine marshes. Along the coast of Campania there are numerous malarious districts especially at the southern part of the Gulf of Gaeta, around the mouth of the Garigliano and in the valley of the Volturno. After we pass the Gulf of Salerno we find malaria in the region where was situated the ancient Pestum. Along the western coast of Calabria as far as the Strait of Messina are found numerous malarious regions. Along the shores of the peninsula bathed by the Ionian sea malaria is most

intense in certain parts of the Gulf of Squillace and of that of Taranto. In Apulia and in Abbruzzo and Molise malaria is found especially in the provinces of Lecce, Foggia, Campobasso, Chieti, and Teramo on the coast as well as in the interior. Of the larger Italian islands Sardinia is full of districts where malaria prevails to a grave extent, these being on the coast as well as in the interior and especially in the southern part. Sicily is less malarious than Sardinia, but we do find here also cases of pernicious infection both on the coast and in the interior.

In Spain and Portugal malarial fevers prevail on the coast and in the plains watered by the Guadalquivir, the Guadiana, the Tagus, and the Mondego rivers.

In *Asia* the shores of Asia Minor, many parts of the Arabian coast, and places on the Persian Gulf are infested with malaria which extends also into Beloochistan and Afghanistan. Malaria is severe on the western coast of India along the course of the Indus, and there is also a region of grave malaria extending from the southern foothills of the Himalayas to the basin of the great delta of the Ganges, and passing backwards along the valley of the Brahmapootra. The island of Ceylon is malarious along the seashore and also in the interior sometimes at a considerable elevation. The plains of Farther India, in which large rivers course and where there are marshes and rice-fields, are equally malarious. Among the islands of the Malay archipelago the most malarious are Sumatra, Java, Borneo, Celebes, and Malucca, but the Philippines are not specially malarious. Malaria prevails in a great part of China, chiefly on the coast, but also in the interior along the courses of the large rivers and in the cultivated plains and rice-fields. The coast of the Corean peninsula is less malarious, and Japan has but little malaria and that little in a mild form.

Africa includes extensive areas of virulent malaria, the worst of which are on the west coast, especially between the Senegal and the Congo, the disease existing in very severe form in Senegambia and in the Niger delta. From the mouth of the Congo downward malaria diminishes in prevalence and in intensity. The region of the Cape of Good Hope is free as is also the island of St. Helena, while Fernando Po and St. Thomas are malarious. Off the southeast coast the islands of Madagascar, Mayotta, Nessi Be, Réunion, and Mauritius are malarious and gravely so. The east coast of Africa is less gravely infected. The coast is malarious from Delagoa Bay upwards as far as opposite the island of Zanzibar, but the Somali peninsula and also Abyssinia are relatively free. The western shore of the Red Sea, including Massowah, is little or not at all malarious, but between

Abyssinia and Lake Tchad is an extensive region of Central Africa more or less malarious, which embraces Nubia, Darfur, Kordofan, and a large part of the Soudan. Upper Egypt is immune from malaria, while lower Egypt, including the valley of the Nile and especially the Nile delta, is malarious. The coast of Tripoli, some parts of Tunis, and especially Algeria are very malarious. It was in the last-named country that Laveran discovered the parasite of malaria which he found there in all the forms of the disease.

In *America* it is especially in South America that malaria prevails. The coasts of Colombia and Venezuela, Guiana, the northern part of Brazil, and the coasts of Ecuador, Peru, and Chili are extensively malarious. In Central America the Atlantic coast is especially unhealthy, that on the Pacific side presenting only here and there a few circumscribed areas of malaria. The disease prevails all along the shores of the Gulf of Mexico and extends up the valley of the Mississippi and along its tributaries. Texas, a part of New Mexico, Florida, and Georgia contain malarious regions. Malaria prevails along the coasts of South Carolina, North Carolina, Virginia, and Maryland, but to a lesser degree in the central and northern parts. There is considerable malaria in southern Michigan and along the shores of Lakes Ontario and Erie, less on the shores of Lake Huron, and scarcely any on those of Lakes Michigan and Superior. In Pennsylvania and New York there are few centres of mild malaria. Canada is almost exempt. There is but little malaria, and that of mild form, on the Pacific coast. The West India islands are very marlarious, but the Bahamas least so.

Australia and also most of the islands of *Oceanica* are almost exempt from malaria, offering in this respect a singular contrast to the lands nearest to them which are so gravely infested. There is malaria, however, on the coasts of New Guinea and of the other islands comprised in the Bismarck archipelago. In New Caledonia, although marshy regions abound, malaria is unknown as it is also in New Zealand and other islands of Polynesia.

Now what are the conditions in these regions of malaria mentioned in this cursory sketch of the geographical distribution of the disease, conditions which do not exist in other regions, and through which is formed what we call the malarious environment? If, as everything leads us to believe, malaria develops in man only through inoculation with the germs made by certain species of mosquito, the study of the conditions of a malarial endemic is much simplified, being reduced to a study of the conditions favoring the life and development of the malarial mosquitos.

The study of malarious regions was not neglected, as we have

said in the section on History, even in the most ancient times, for we have examples of sanitary works dating back to those eras which show that the ancients were not ignorant of the conditions favoring endemic malaria. In a more recent period our endemiological knowledge was amplified and perfected especially by the works of Morton and Lancisi. The conditions favoring the prevalence of malaria in any region are climatic and telluric.

Climatic Conditions.

Among the climatic conditions the one of most importance is heat. The part played by heat in the production of malaria is undoubted; we have only to consider that malaria rarely extends beyond 63° to 64° of north latitude and 57° south latitude (Hertz), and that in proportion as we pass from these limits towards the equator the disease progressively increases in prevalence and virulence. Hirsch (quoted by Hertz) sought to determine exactly the northern limit of malaria, but found in individual malarious centres great differences in temperature and latitude, and demonstrated that it was not the mean annual temperature that should be taken into account, but the mean summer temperature. He places the northern limit of malaria between the isothermal lines of 15° and 16° C. (59° and 60.8° F.).

The importance of heat results also from the fact that, while malaria in the tropics where it prevails endemically presents merely oscillations in relation particularly with the dry and rainy seasons, in temperate regions it is especially in the summer and autumn that the disease prevails, and again in those parts where all the varieties of malaria are found it is only at these seasons that we see the grave and pernicious forms of the disease. Thus in the malarious parts of the Roman Campagna malaria occurs only in its milder forms (chiefly simple tertian) in the spring, while the grave forms constituting the estivoautumnal endemic begin after the first extreme heat of summer, usually in the first part of July, and continue with oscillations during the summer and autumn, the endemic being more or less prolonged according as the cold comes late or early, but usually ceasing abruptly about the end of December. In winter we observe relapses of the infection contracted in the summer and autumn, and these growing progressively milder usually cease in the spring, but occasionally continue until summer.

From what has been said under the head of incubation, it will be readily understood that the last cases of primary attacks observed at the end of the season of malaria may not declare themselves for many days after the possibility of infection has passed; and this is the ex-

planation of those cases in which a primary febrile paroxysm occurs after the first frost.

This relation of the malarial infection to the seasons is described by Lancisi as follows: "Itaque principio æstatis febres et plurimum tertianæ non malignæ corripiunt: adaucto vero æstu febres continuæ atque etiam exitiales urgent; longo tamen deteriores evasuræ et plane pestilentes circa æquinoctium autumnale, præcipue si pluviæ, nebulæ, rubigines, ventique australes accesserint. Tandem circa hyemale solstitium de pernicie ubique remittunt; sed in chronicas affectiones abeunt: qui enim ab ejusmodi castrensibus febribus liberantur, fere semper contumacibus viscerum obstructionibus, et quartanis longo dein tempore duraturis divexari solent."

The close dependence of the malarial infection upon the seasons is well shown in the following table, drawn up by Dr. Ballori, showing the number of patients with malaria received each month during the years 1889–96 in the Santo Spirito Hospital at Rome:

	1889.	1890.	1891.	1892.	1893.	1894.	1895.	1896.	Total.
January	68	71	35	68	113	171	160	220	906
February	44	56	33	24	50	67	96	168	538
March	41	53	63	84	70	74	92	164	641
April	35	46	64	79	48	34	119	130	555
May	80	60	72	204	86	75	148	161	886
June	81	82	113	212	76	81	182	101	928
July	698	674	420	402	695	807	833	205	4,734
August	1,249	1,325	587	478	589	924	942	713	6,807
September	890	561	409	267	659	870	804	437	4,897
October	371	369	114	190	434	559	674	415	3,126
November	256	303	102	177	319	477	547	200	2,381
December	123	200	83	227	150	185	171	137	1,276
Total	3,936	3,800	2,095	2,412	3,289	4,324	4,768	3,051	27,675

This table shows that most of the cases of malarial fever occur in the months of July, August, September, October, and November. In December the number of those received for malaria is markedly less, and continues to decrease progressively through the winter, during which time only patients with relapses are received. In the months of May and June there is a more or less noticeable increase marking the spring endemic, but there is a striking and sudden increase in July denoting the beginning of the estivoautumnal endemic.

This relation between the number of cases of malaria and the months of the year is not the same everywhere. In some malarious districts in Italy the maximum of the estivoautumnal endemic occurs in the fall, especially in September and October, and in other places even in November and December. Furthermore, in the same place

the period of maximum prevalence may vary in different years. In the Roman Campagna, for example, in 1898 the greatest number of pernicious fevers occurred in November and December and even in the first part of January.

It has been said that heat is a very important factor in the development of malarial endemics, but as we shall see later the effect of high temperature does not declare itself immediately, and there are other intermediate factors concurring in the production of malaria. Thus, while it is certain that the estivoautumnal endemic always develops after the first strong heats of summer, it is also certain that it may be prolonged in the late autumn when the temperature is lower than that of June in which few or no cases of primary estivoautumnal infection are seen. Furthermore, the gravity of the malarial endemic in any year is not always, as Celli has pointed out, in direct ratio with the height of the mean temperature for the warm months. In fact, as Celli likewise notes, in 1879, which was a year marked by a very severe endemic outbreak of malaria, the mean temperature for July and August was lower than it had been for eight years.

With this course of the malarial endemic relative to the seasons, we may compare that of the life of the malarial mosquitos. With the first intense cold some of the mosquitos die, others hibernate. In the spring the latter emerge from their hiding-places and deposit in stagnant water their eggs, whence come the new generations to which others succeed in the summer and autumn. Further studies, which are now in progress, will doubtless reveal the relationship between the development of the estivoautumnal endemic and what happens to the mosquitos whereby they become infected. From certain researches made last year we have learned that in the late autumn the malarial mosquitos, very many of which are infected, seek shelter in the houses. This explains the great liability to infection at this time and also the curious fact of true house epidemics of malaria.

We have said that as yet an explanation is wanting of the origin of the estivoautumnal endemic and is even now being searched for from many sides. But we may be permitted to hazard some conjectures: Have the daughter mosquitos inherited from their mothers the infection which requires an elevated temperature in order to develop and be transmissible to man? Do the daughter mosquitos infect themselves by sucking blood still containing crescent forms, and then, do these develop in them later when the temperature of the air permits? Or do the mosquitos become infected in some other way, as, for example, by sucking the blood of other animals? Doubtless the true solution of this problem will soon be forthcoming. In the mean while we must not omit to mention that some, Celli for example,

admit the possibility of a seasonal polymorphism of the hæmosporidia of human malaria, that is to say, of the transformation of the spring parasites into estivoautumnal parasites.

Rain is recognized by all as an important factor in the production of malarial endemics. In tropical countries, where the rainy season alternates with the dry, we find the curve of malarial morbidity corresponds very nearly to that of the rainfall in such a way that the maxima of the first follow those of the second at an interval of about a month. Plehn has observed such a correspondence in Kamerun and he publishes a chart which shows it very clearly.

In temperate climates it is held that a very rainy spring is followed by a malarial season more serious than usual by reason both of the number of cases and of their gravity; and also that the summer rains as well as those of the autumn have an injurious influence.

The relation between rain and malaria is not very simple and cannot be easily shown by statistics. How may the rain influence the development of malaria? Certainly in two ways: Firstly, by favoring the telluric conditions necessary to the production of malaria, or, more exactly, of the life of the malarial mosquitos; secondly, by acting as an occasional exciting cause of the development of the infection in the human organism.

The abundant rains of the spring cause the formation of numerous stagnant pools and marshes where the mosquitos can deposit their eggs, and similarly the rains of the summer and autumn keep these pools and swampy places from drying up. But the amount of rain must not exceed a certain limit, for when heavy showers follow each other at short intervals, the exit of the winged insect from the puparium may be prevented. But the influence of rain in the production of malaria may be nullified by several factors, as, for example, when a strong wind is blowing in the intervals of the showers so as to dry the soil quickly. To this action of rain we may, as above said, add another, namely that of favoring the manifestation of the infection in a person who already has the germ in his body. This second effect of rain is manifested very quickly, while the first mentioned requires considerable time—a month according to experience in the tropics, which is a period corresponding to that of the aquatic life of the mosquito plus the period of incubation of the disease in man.

Winds were formerly regarded as carriers of malaria from one place to another, but all are not now agreed as to this action. Some would have us believe that malaria may be transported long distances, even from one continent to another across the sea, by the agency of the wind; but others have denied this possibility, and others again

regard the wind as a factor of salubrity by dispersing and destroying the malarial germs. The first of these opinions was held by Lincisi, who, in accordance with this belief, would not permit the cutting down of groves, even in the plains, maintaining that they acted as filters purifying the air passing through them of the emanations from marshes. But this view is opposed by so many and such convincing facts, related by many observers, among others and especially by Tommasi-Crudeli, that we can no longer admit any connection between winds and the diffusion of malaria. It is sufficient to recall the fact that on ships anchored even close to the shore of exceedingly malarious districts, the members of the crew never suffer from malaria so long as they remain on board the vessel, but that many acquire the disease and perhaps succumb to it if they go ashore and remain even for a single night. Thus, Vincent and Burot (cited by Mannaberg) state that in the Madagascar campaign of 1895, while the French troops were decimated by the fevers, the sailors who remained for months on board the ships hardly three hundred yards from the shore escaped. Then again, in very circumscribed malarious districts the infection may be intense, but it will remain within these narrow limits for years and years without spreading in any direction; and in cities like Rome, situated in the midst of an eminently malarious region, the inhabitants never have malaria, even during the season when every day numbers of fever sufferers are brought to the hospitals from the neighboring Campagna.

If it is true that man acquires malaria only through inoculation by infected mosquitos, then the habits of the latter will explain why, as a rule at least, malaria cannot be transported by the wind. For as a matter of fact, when the wind blows the mosquitos conceal themselves in the grass or on the leaves of the bushes, and only when the wind dies down in the evening do they take wing, sting men and animals, and invade the houses. But, more than this, the observations of Grassi demonstrate positively that malarial mosquitos are not transported by the wind, for he found Anopheles in circumscribed malarious districts, but was unable to discover any at all in neighboring fever-free regions. We also have made repeated and careful search in the gardens within the city of Rome, situated at a very short distance from the malarial Campagna, but we have never succeeded in even a single instance in finding any specimens of Anopheles.

Telluric Conditions.

Up to within a short time ago it was thought that malaria was of telluric origin and that the pathogenic germ rose into the air from the soil and from stagnant pools of water; and this belief gave origin to the study of the soil in malarious regions, a study which was prosecuted in all directions; the geological nature of the soil in places where malaria exists was determined, a search was made in the ground for the malarial parasite, and the endeavor was made to discover in what way the germs were carried a certain distance above the ground especially at given hours.

The geological character of the soil is of no particular importance; calcareous, argillaceous, and even granitic soils may be found in malarious regions. It is not the nature of the soil that exerts an influence in the production of malaria, but the fact that beneath a more or less thick stratum of humus there is an impervious layer, for example, of calcareous tufa, marl, or clay; the consequence of this is that the soil is permanently moist and there is a layer of water at the bottom of the permeable layer while there are pools formed in the depressions of the surface. Such conditions exist throughout the Roman Campagna. To demonstrate the importance of this factor we will recall the example given by Meunier (quoted by Hertz) of what took place when the necessary excavations were made for the railway from Madrid to the Escurial. For a distance of fifty kilometres from Madrid no cases of fever occurred among the laborers, but in the construction of the second half of the line the workmen suffered severely from malaria. There was no difference whatever in the hygienic conditions, but there was a difference in the nature of the soil, which was for the first half diluvial and sandy, but granitic and schistous for the second half of the distance.

That the production of malaria is related to the humidity of the soil and to the presence in it of collections of water, is demonstrated by the fact that malarial endemics exist by preference in low marshy places, in the deltas of large rivers, in the broad alluvial plains bordering wide rivers, and in valleys in which are swamps and water-courses. When malaria exists also in places of greater or lesser elevation, we find in them, or rather in the tablelands there located, the same conditions of soil formation as in the malarious plains below.

The ancients attached the greatest etiological importance to marshes, and we have already seen that Lancisi recognized as the only cause of intermittent fevers the noxious effluvia rising from swamps. He made a distinction between noxious marshes and those of a harmless character. The noxious swamps were those of wide

extent and shallow, in which, although there might here and there be an intermittent current, for the greater part of their circumference, especially where the banks were flat and covered with rank vegetation, the water was stagnant; it was from the death and decomposition of the myriads of insects and of the marshy vegetation that the noxious effluvia arose. The harmless bogs were those in which the water, either fresh or salt, was for the most part deep, in constant motion, containing little slime, with many fish, and especially if the banks were high and not grown over with canes and reeds. Lancisi says that he had seen malarial endemics disappear after the drying up of bogs. He records an instance in point: As a sequence of the formation of a marsh in Rome in the Celimontana valley near the church of San Giovanni in Laterano there occurred an endemic of fever and a plague of mosquitos, and in the neighboring hospital of San Giovanni there were several cases of pernicious fever that summer; but the endemic ceased when, upon Lancisi's advice, the marsh was filled up.

But, although swamps are not infrequently found in malarious places, it is not correct to assume that the presence of marshes and the occurrence of malaria are two closely connected facts, for there are marshy places where malaria does not exist and malarious districts which are not swampy. If, as Tommasi-Crudeli observes, the malaria in the Roman Campagna were dependent upon the presence of swamps, it would prevail over a very limited area. But the same author remarks that, in malarious districts where no swamps exist, there are to be found many small collections of water, little ponds and pools all about, fed by the rains or by the subsoil water. These collections of water are more than sufficient for the development of mosquitos, especially the malarial varieties, which require stagnant pools surrounded and covered with vegetation.

In addition to these swamps, stagnant pools, etc., the occurrence of malaria may be favored by the presence of rice-fields, of places where hemp is macerated, and of ponds on the seashore where the salt water is mixed with the fresh. Even irrigation of cultivated fields may be a cause of malaria, and the history of irrigation in Southern California has made it plain that if irrigation works are not to become producers of malaria, drainage must proceed *pari passu* with the irrigation; when this is not done, the water which brings riches brings also malaria.

Anything which results in the formation of pools of stagnant water, such as inundations, the denudation of hills, ploughing, and in general any upturning of the soil in the construction of railroads, canals, fortifications, diking of rivers, etc., may be productive of a

malarial endemic. Inundations have sometimes been followed by a recrudescence of malaria in places where the disease had formerly existed. Frerichs, in 1854, observed an endemic of grave and even pernicious fever, following an overflow of the river Oder in Silesia, where before there had been cases only of mild malaria.

While the cutting down of trees in the plains may contribute to the sanitation of such places, the same thing in the uplands may, in consequence of hydraulic disturbances thereby produced, be a cause of aggravation of malarial endemics. This happened, according to Pellarin (quoted by Rho) in the island of Mauritius, where, after the denudation of the hills, the little mountain torrents which formerly ran down to the sea now disappeared on the way and ended in pools of stagnant water, while in the rainy season they often overflowed the surrounding country, forming temporary marshes. Coincident with these telluric changes the malaria on the island became more widely spread and graver, and cases of pernicious infection occurred. The remembrance is still fresh of an endemic of malaria occurring in the Trastevere quarter of Rome when the works preliminary to the banking of the Tiber were begun. Even the works undertaken for sanitary and economic purposes may be the cause of an outbreak of malaria or of an aggravation of the already existing endemic. Many of those upturning a virgin soil have paid tribute of health and even of life to the fever goddess, as has been seen in America, Algeria, and many other parts of the world.

It is said that malaria prevails chiefly in plains and valleys, and to this we may add that the frequency of its occurrence diminishes with elevation above the level of the sea, and even immediately above plains made desolate by malaria salubrious regions are often found. Thus the district of Norma on an abrupt rocky elevation about four hundred metres above the Pontine marshes enjoys a most salubrious atmosphere. Malaria may, however, exist in the mountains; for example, on the eastern slope of the Rocky Mountains it is found at an elevation of 2,000 metres (6,500 feet) and in the Peruvian Andes at 2,500 metres (8,125 feet). In Italy malaria may also be found at considerable elevations; thus Grassi has recently discovered a malarious district near Colico at a height of 2,600 metres (8,450 feet).

The study of circumscribed malarial endemics is very instructive as showing us the telluric conditions necessary to their existence, and also certain characteristics of malaria of the greatest practical interest. There are many examples of very circumscribed endemics in medical literature. We will relate one important instance studied by Spadoni and ourselves near the city of Senigallia on the Adriatic. In this city, renowned for its beautiful shores and for the green

and smiling hills about it, in which are schools, hospitals, and other public institutions, and which is visited in the summer by a numerous colony of bathers, malaria is unknown, as it is also in the surrounding country and in the neighboring hills where there are numerous cottages, villas, and houses inhabited the entire year. But there is here one very limited area of malaria which is quite grave because of the number of cases as well as of the severity of the disease in those attacked. This endemic is situated without the walls in a little suburb consisting of a row of houses, in some places double, along the left bank of a large drainage canal constructed for the purpose of carrying off the excess of water coming from the hills during heavy rains and conducting to the sea the overflow from the river Misa. During the hot season the flow of water in this canal ceases, but in consequence of irregularities in its bed and banks there remain numerous pools and stretches of stagnant water the surface of which is covered with aquatic plants. The canal is deep, and in the upper part its irregular banks are covered with vegetation, while in the lower part its bed widens out as it nears the sea. Spanning the canal are four bridges, the one nearest the sea being for the railroad. Now the malarial endemic is confined to the houses situated along the upper part of the canal, very many of the inhabitants of which are sufferers from malaria, those sleeping in the lower stories being first attacked and then those in the upper rooms whose windows look out on the canal. We note especially this last particular, for no cases of malaria were found among those who lived in the upper rooms facing the street which runs parallel to the canal behind the first row of houses. One very interesting observation should be recorded. A family of six persons occupying the upper story of one of the houses on the bank of the canal, the windows of which, however, looked on the street, had remained free from the disease; but early in August, 1897, this family was obliged to remove to the lower floor of the same house, on the canal side, and after having been there fifteen days every one of the six members came down with malarial fever. The new tenants of the upper rooms which had been vacated by this family remained in good health.

We have said that the houses along the canal form a double row at some points, while at others there is but a single row, this being sometimes on the canal side of the street, sometimes on the other side. Now, where the row on the canal side is interrupted the inhabitants of the other row suffer from malaria, but where there are rows on both sides those in the houses on the farther side, which are sheltered from the canal, escape.

With the exception of the houses mentioned there are no others

visited by malaria either in the surrounding country or in the city or in the hills; and even, as we have just said, there is no malaria in the rooms of the houses along the canal the windows of which look out on the street behind. We must note also that there is no malaria in the houses along the lower part of the canal where the sea enters for a short distance; indeed, some of these latter houses are even rented by the summer visitors. Nor is there any malaria along the line of the railroad nor beyond it among the laborers in a large sugar refinery.

The malaria of this well-defined endemic occurs in all its forms. In the spring we find only the distinctly intermittent fevers, especially tertian, but in the summer and fall the estivoautumnal infection predominates, and cases of pernicious fever also occur; in winter there are the relapses, the anæmic and the other sequelæ of infection. The various febrile types are encountered among those occupying the same house and even the same room. A microscopical examination of the blood reveals the presence of the various species of malarial parasites corresponding to the clinical forms observed.

From the description just given of this markedly circumscribed endemic of malaria, in which we find all the forms of infection, it appears most clearly—as also in other centres of grave malaria, the Pontine marshes for example—what are the telluric conditions necessary to its development and maintenance. These conditions consist in the formation, during the summer, in the bed and banks of a canal, of small collections of stagnant water covered with a layer of vegetation, surrounded by mud and shaded by the rank vegetation of its banks. Here are no extensive marshes, nor even stagnant ponds, but only puddles in which plant decomposition takes place. Now, in these stagnant pools the larvæ and nymphæ of the mosquitos which infest the houses of the wretched sufferers from the fever find a suitable nest. That the telluric conditions mentioned were the true determining cause of the malaria was demonstrated by the fact that this latter, running into the autumn of 1897, did not reappear in the summer of 1898 when these conditions had been removed by a process of natural sanitation. Torrential rains and floods occurred throughout that region in the autumn of 1897, carrying away much of the sediment in the canal, deepening it by from two to four metres and uncovering the timbers of the old bed; the flood also wore away the banks smooth and even undermined some of the houses. The consequence of this was that in the summer of 1898 the water of the sea entered the canal which was kept clear by the ebb and flow of the tide. Not a single case of fever occurred among the occupants of the houses infested by malaria during the previous

years; the mosquitos also almost entirely disappeared, and the few specimens caught were all of the genus Culex.

From the study of this circumscribed endemic another very important fact is developed, namely, that endemic malaria remains fixed in the place where the telluric conditions are favorable and does not spread to any distance, even horizontally. Indeed, this intense and grave malarial endemic was confined for years and years to the few houses whose doors and windows looked out on the upper portion of the canal where the stagnant pools were located. One gate of the city is but a short distance from the canal, and from the same part is seen one of the bridges crossing it; immediately within the gate is an asylum founded by Pius IX., the hospital, and many houses, in all of which malaria is unknown; and of the city guards who are stationed at the gate from early in the morning to late in the evening none can be found who has ever suffered from intermittent fever. It is evident from this that malaria is not carried by the winds but that it is joined to the conditions of the place where it is located, and that those are right who maintain that, instead of endeavoring to secure protection from unhealthy winds, it is necessary to attend to the sanitation of the soil.

We learn also from this instructive instance that the drinking-water cannot be the vehicle of infection, for those who suffered at Senigallia drank the same water as the occupiers of the immune houses.

We have scarcely touched upon the meteorological and telluric conditions noted in places devastated by malaria. But the study of these, especially of the second named, will be much easier in the future, for, as it is now known that the malarial parasite does not live free in the soil or in the water, but that it enters the bodies of certain species of mosquitos, the problem will be greatly simplified. Guided by the two grand discoveries, that of the parasite in the blood and that of the malarial mosquitos, not only shall we be enabled to describe more clearly the endemiology and the geographical distribution of malaria, but we shall also know better what to think of malarial pandemics, and shall learn why in certain places, where all the conditions favorable to the development of malaria appear to exist, the disease is actually unknown.

GENERAL PATHOLOGY.

Changes in the Red Blood Corpuscles.

The changes which occur in the blood in malaria are of two kinds, the first being due to the direct action of acute malarial infection and involving both red and white corpuscles (pigmented globuliferous

parasite-infected leucocytes), the other being secondary to the anæmic condition which is the result of the parasitic invasion. Among the first, the most important are the lesions of the red corpuscles caused by the action of the parasites which develop within them and which are nourished at their expense. Some of these lesions differ according to the kind of malarial parasite, and of these we have already spoken when describing each of the varieties of parasites, calling attention to the fact that the gravest lesions are found in estivoautumnal fevers. There is, however, one alteration which, with rare exceptions, is constant in every kind of human malaria, and common to all, and this is melanæmia.

The other lesions of the red cells, enumerated above, are of great interest in relation in the pathology of malaria, for which reason we will briefly enumerate them here:

Swelling of the Red Corpuscles.—This is found especially in the ordinary tertian; the red cells are invaded by parasites, and gradually increase in size until they are two, three, or even more times the usual size; at the same time they gradually lose their color until they finally become very pale, so much so that sometimes the corpuscles containing adult parasites, especially the forms known as gametes, are scarcely to be recognized by their outlines. They frequently are changed in shape, becoming more or less oval. In fixed and stained specimens we sometimes find that adult parasites which seem to be free in the plasma are really contained within corpuscles which have lost their hæmoglobin.

Shrinkage of the Corpuscles with Changes in Color of Hæmoglobin.—This change is best exemplified in the so-called brassy bodies described by Marchiafava and Celli, found, as we have already mentioned, in the summer-autumn fevers, and only occasionally, as Bastianelli and Bignami have noted, in the ordinary tertian. The red corpuscle takes on the color of old gold or of brass, becomes smaller, and shrivels, so that the lesion may be briefly designated as *erythropycnosis.*

Brassy bodies are found under various conditions. Above all are they met with in the apyrexia preceding a fresh febrile attack (Marchiafava and Celli), when all or nearly all the estivoautumnal parasites in the circulating blood have become pigmented at the periphery or have pigment in the centre or a little excentrically. They may also be found after the administration of quinine, in which case many of the red corpuscles containing young, non-pigmented parasites are also brassy (Marchiafava and Bignami). In this latter event, we must believe that quinine in its final action determines a necrosis not only of the parasite but of the red corpuscle containing it, and in

fact, after the lapse of a certain amount of time all these bodies disappear from the blood. Quinine does not, however, cause these changes in all parasite-infected corpuscles, for it not rarely happens that after its administration many free parasites are found in the blood, evidently having come out of the red corpuscles; this may be well observed in specimens prepared by Romanowsky's method.

It is more difficult to establish with any certainty what becomes of the parasites contained in the brassy bodies in the first class of cases—that is to say, previous to a febrile attack, independently of the action of quinine. As we know that all or nearly all the parasitic forms which reach the stage of multiplication are found stationary in the viscera, we hold it to be almost a certainty that the adult forms circulating within brassy bodies die with the corpuscle containing them; in other words, it seems to us probable that the necrosis of the corpuscle prevents the further development of the parasite. It is true that we sometimes see parasites which have completed fission within brassy bodies, but it seems to us likely that in such a case the corpuscle has not long been brassy, but that the alterations in its condition occurred after the complete development of the parasite. We therefore hold that this "erythropycnosis" represents a necrosis of the red blood corpuscle, followed, in all probability, by the death of the included parasite.

Partial Decolorization of the Red Blood Corpuscles.—This is a change which is not infrequently met with in estival fevers. In some red globules containing bodies with blocks of pigment, we find the hæmoglobin collected and as it were condensed around the parasite as though attracted to it, while the remainder of the corpuscle is seen to be more or less decolorized and usually shrivelled and wrinkled. This same condition of things is often found in the crescent bodies, which are surrounded by a thin layer of hæmoglobin forming a sort of membrane around them, the rest of the corpuscle being recognizable only by its very faint outline.

In some cases, in which two bodies with central pigment masses were included within the same blood corpuscle, we have seen the hæmoglobin forming a sort of halo around each one, the globule being perceptible only by the delicate line which indicated the periphery. This would lead us to believe that at the periphery of the corpuscle there is a stratum of tissue differentiated from and more resistant than the discoplasm, and forming a sort of membrane. In some cases indeed the parasite appears to be confined within a sort of bag, which is not well filled, and whose walls are withered and wrinkled. When an adult parasite comes out from a corpuscle, or a fission form is set free, and the spores disperse, this peripheral stratum appears to

burst; at the same moment the hæmoglobin is lost in the plasma. It may be due to the presence of this resistant peripheral layer that the pseudopodia (even the large pigmented pseudopodia of the tertian parasite) do not project beyond the surface of the red corpuscles.

Fragmentation of Parasite-Infected Corpuscles.—This is not of frequent occurrence. We have occasionally seen a corpuscle containing, for instance, a pigmented estival body, divide into two parts, forming two little corpuscles, one of which contains the parasite (parasitiferous schistocytes).

Changes in the Physical Properties of the Parasite-Infected Corpuscles.—Great importance has been attributed to these changes occurring in grave fevers as thereby the stagnation of the corpuscles in certain vascular areas is explained. Bignami, when studying the distribution of the parasites in the vessels of the various viscera in pernicious fevers, noticed that the parasite-infected red corpuscles in the veins of a certain calibre showed a tendency to place themselves against the walls of the vessels, and that sometimes they would gather in one vein, being grouped together as if agglutinated. This circumstance he endeavored to explain by assuming a diminished elasticity in the discoplasm, and a qualitative alteration in the surface which had apparently become viscous. Owing to this fact, red corpuscles containing summer-autumn parasites, especially when these are adult bodies, are less adapted than normal ones to the circulation.

The truth of this statement can be proved by direct observation. If in an ordinary fresh preparation of estivoautumnal blood in which are numerous corpuscles containing bodies with blocks of pigment we cause a current by pressure, we shall see that the corpuscles containing the above-mentioned bodies scarcely move, and appear almost to cling to the glass. It is therefore natural to suppose that many of the corpuscles, even those which present no alterations recognizable under the microscope, are less elastic and more viscous than the normal.

In only two cases of hæmoglobinuria Bignami noticed a tendency to agglutination on the part of red corpuscles which were not infected by parasites.

Melanæmia.

As we have already stated, the most characteristic alteration in malarial blood is melanæmia, and this it is which from the earliest days of modern research has attracted the attention of investigators. For this reason the history of the knowledge so far obtained is of the greatest value in giving us an insight into the progressive development of the researches regarding malaria.

Melanæmia, so far as is known at present, occurs only in malarial

infections, and is one of the most characteristic alterations of this infection. It consists in the presence in the blood of a pigment of a brownish, or black, or brownish-yellow or reddish-brown color, which occurs in the form of granules, rods, needles, or blocks, the joining together of which gives conglomerations of greater or lesser size. In rare cases they are free, but as a rule they are included within the body of the malarial parasite or in the leucocyte. It is easy to determine the existence of the pigment in the blood by examining a thin layer under the microscope—indeed, previous to the discovery of the parasite this was the chief method in use for establishing the diagnosis in doubtful cases.

History.—From the very beginning of the researches in regard to this pigment, nearly all writers agreed in the belief that it was derived from the coloring matter of the red blood corpuscles, but many were the discussions as to the place of its formation and the method by which this took place. As early as the last century some physicians observed that various organs were of a black or dark appearance in grave malarial fevers. Thus Lancisi,[1] in speaking of the changes found in those who had died from malaria, says: "Primum in iis qui ob tertianas perniciosas occiderunt, ingens malorum sedes sub aspectum venit in abdomine, ubi omnia livida, et potissimum hepar subfusci, ac bilis cystica atri coloris passim occurrerunt."

Meckel was, however, the first to observe the particles of black pigment in the blood, and he held that they had come there from the spleen. Tigri also observed the melanosis of the spleen, and gave it the name of black spleen.

Virchow,[2] having noticed numerous pigmented cells in the blood and spleen of a man who died with dropsy, after many attacks of intermittent fever, supposed that the pigment originated in the spleen. To Virchow and to Frerichs we owe the theory that melanæmia represents a dyscrasia due to the alteration of some organ. To Frerichs[3] we owe an accurate description of melanæmia and of its effect upon the organism. He observed in the blood free black granules and molecules, and pigmented cells resembling leucocytes, now fusiform and now cylindrical in shape; he moreover described accumulations of black granules held together by a pale substance, or having an involucrum of a hyaline substance which was sometimes thin and sometimes thick.

As to the place of formation of the pigment, Frerichs held that it was the spleen, for the following reasons: (*a*) Pigment is found in the normal spleen; (*b*) in melanæmia there is always more pigment in the spleen than in the general circulation; (*c*) in the general circulation we find pigmented splenic cells. He believed, moreover, that some-

times even the liver might participate in the formation of pigment. As to the method of its formation, Frerichs thought that in malarial hyperæmia of the spleen the blood was poured in large amount into the lacunæ of this organ, stagnated there, and was there destroyed, whence the formation of masses of pigment from the hæmoglobin of the red corpuscles. That this formation of pigment does not occur in hyperæmia of the spleen from other causes is because chemical changes of the splenic juice are produced in malaria which menace the existence of the red blood corpuscle. Frerichs, therefore, agrees with Virchow that in intermittent fevers the pigment is formed in the spleen, and then enters the circulation.

A good description of melanæmia is given by Forsyth Meigs,[4] who lays stress upon the intimate connection existing between the formation of black pigment and intermittent and remittent fevers, stating that he has looked in vain for the same condition in other diseases. This writer notes the marked diminution in the number of red corpuscles during acute infections, and accurately describes the appearance of the viscera in the cadaver, dwelling upon the characteristic aspect of the nervous centres in which, as a rule, the pigment is found in minute granules within the capillaries, sometimes in such abundance as to modify the color of the nerve substance. "Nothing in pathology," he says, "has surprised and interested me more than this condition of the nerve centres."

The pigment granules, according to Meigs, are found within cells not to be distinguished from leucocytes or splenic cells, but sometimes the pigmented cells have "an oblong or spindle-shaped outline" (Body No. 2 of Laveran?). The pigment is most abundant in the spleen and in the portal vein, but in grave cases it is found in the whole organism. In the splenic pulp the author claims to have found red corpuscles in various stages, not only of disintegration, but of metamorphosis into true pigment; wherefore, with Frerichs he holds that black pigment originates from the hæmoglobin.

Of the various investigators who followed some agreed with the views of Virchow and Frerichs, and others denied them.

Thus, Colin[5] holds that the formation of pigment occurs not alone in the vessels of the spleen, but also in those of other organs; but he also asserts, without giving sufficient reasons for the belief, that this formation of pigment has nothing specific in its nature, because it occurs in other diseases as well, for instance, in the mesenteric glands in typhoid fever and in dysentery. In malarial infection, however, he holds that the condition is more conspicuous than in other diseases, because the destruction of red corpuscles is more rapid and more marked.

Mosler[6] leans towards the belief of Virchow and Frerichs that the primary formation of the pigment is in the spleen. He holds that the special structure of the spleen lends itself to this formation of pigment —that is to say, that the blood flowing from the capillaries into the intermediate blood-vessels not rarely stagnates there, so that conglomerations of red corpuscles occur which gradually become converted into pigment. He moreover believes, with Frerichs, in a chemical change in the quality of the splenic juice in malarial enlargement.

A notable advance in the study of the question is marked by the researches of Arnstein,[8] who maintains that the pigment is formed in the circulating blood during the febrile attack, and is deposited by it in the spleen, liver, and bone marrow. He observes that the pigment is found in the blood free or included in white corpuscles (which is the usual occurrence) during the fever or shortly after. On examination of such organs as are most melanotic (the spleen, liver, and bone marrow), he finds that they contain pigment not only in the blood-vessels, but also around them, and only in cases of recent infection does he find it in other organs, such as the brain and kidneys. Arnstein holds that the theory of Virchow and Frerichs is not tenable, but believes that the melanæmia (the presence of black pigment in the circulating blood) is the primary occurrence, and the melanosis of the spleen and liver secondary; indeed, melanæmia may be found for only a short time after the febrile paroxysm, which would not be comprehensible if the melanosis of the spleen were primary. And finally the disposition of the pigment in the circulating blood corresponds perfectly with what occurs when coloring matter (as cinnabar) is introduced into the circulation.

According to Arnstein, then, during the febrile attack the red blood corpuscles are destroyed, and the pigment which is formed is rapidly taken in by the leucocytes which stagnate in the veins and capillaries of those organs in which the circulation is slowest—that is to say, the spleen, the liver, and the bone marrow—whence the pigment is deposited in the tissue of these organs. As to the mode of formation of the pigment, Arnstein acknowledges that he knows nothing, not having been able to follow the process of disintegration of the red blood corpuscles through all its stages. He holds it to be probable that the pigment is formed in the blood serum from hæmoglobin which has come out of the red blood cells. He does not believe that the pigment is formed within the leucocytes, as Langhaus observed in hemorrhages, because we find free pigment in the blood and no globuliferous cells. These, however, are found in the spleen and in the bone marrow, but they are few in comparison to the enormous amount of pigment found in the circulating blood.

Arnstein's conclusions were promptly contradicted by Mosler[7] in a later work, and upheld by Kelsch[9] who carried out such accurate researches that they can be read with profit at the present day.

Kelsch, making use of a rich amount of material found in the hospital of Philippeville, describes a series of observations upon malarial anæmia and melanæmia. Of the former we will speak presently. As to the melanæmia, Kelsch describes in the blood of malarial patients, especially those suffering from pernicious fevers, the presence of free pigment, or pigment included in hyaline masses, or more often still in white corpuscles. He notes that there are mélaniferous elements which contain pigment granules arranged in wreath form; he describes others which give a brownish reflection in the marginal zone, and contain fine black granules; and in the blood of the splenic and portal veins he found melaniferous cells which were most varied in form and size, being spherical, polyhedral, ovoid, elongated, biscuit-shaped, etc. As to the distribution of the pigment, from a study of the various organs in patients who died of pernicious fever, Kelsch comes to the conclusion that it behaves exactly in the same way as granular coloring-matter injected into the circulation. In opposition to the theory of Virchow and Frerichs, he believes that it is formed in the circulating blood; in fact, in a case of fulminating pernicious fever he found little pigment in the spleen, while the blood was filled with it. As to the mode of formation, he cannot admit that any of the elements represent the stroma of decolorized red cells with pigment granules formed at the expense of the hæmoglobin, because he did not succeed in finding the intermediate stage of this retrogressive metamorphosis; nor does he admit Langhaus's theory of the intracellular formation of pigment, because the pigment is also found free in the blood. He takes refuge in the hypothesis that the melanotic material proceeding from the destruction of the red blood corpuscles exists in the serum in a state of solution, and when the blood becomes saturated is precipitated in the form of granules which are speedily taken in by the leucocytes. This is what occurs when cinnabar is injected into the blood.

The genesis of pigment within the red blood cells was suspected by Marchiafava[10] as early as 1879. From his study of the splenic pulp and of the bone marrow in melanæmic children he came to the conclusion that the red blood corpuscles do not give rise to the formation of pigment after their disintegration, but that on the contrary the conversion of hæmoglobin into melanin occurs by degrees within the corpuscle itself—a conclusion which has been confirmed by all subsequent research.

Leaving aside the observations of Afanassiew,[11] who, having

doubts as to the origin of the pigment from the red corpuscles, suspected the parasitic nature of the pigment granules, and holding them to be analogous to the Micrococcus chromatogenus of Cohn, we come to the researches of Laveran,[12] Richard,[13] and Marchiafava and Celli,[14] by whom the theory of melanæmia was definitively established.

The first observations of Laveran, which have been referred to in the section on Parasitology, while they led this author to assert the parasitic nature of the pigmented bodies (Bodies No. 1, No. 2, and No. 3) did not lead him to an exact recognition of the genesis of melanæmia. In fact, not having observed progressive endoglobular development of his pigmented bodies, he was inclined to believe at the beginning of his researches that the pigment was an integral part of the parasitic body; so much so that in the waters of malarial regions he sought a pigmented parasite, but not finding it, he advanced the theory that the pigment might have come from a destruction of red corpuscles.

From this long series of researches, to which, for the sake of brevity, we will not add the opinions of the other observers, it seems to us that in spite of all contradictory evidence it has been proved, especially by the observations of Arnstein and of Kelsch, that the black pigment is formed in the circulating blood, and that consequently the melanosis of the viscera (spleen, liver, etc.) is secondary to the melanæmia; but that the genesis of the pigment is still in the domain of theory. Was the pigment formed in the serum from coloring matter which had escaped from the red corpuscles, as Arnstein believed, or did the melanotic substance exist in the plasma in a state of solution and become precipitated when the plasma was saturated? Was it a part of the body of the parasites, or did the action of the latter determine its formation from the red blood corpuscles?

Very evidently no convincing solution of the question could be reached except by a methodical study of the alterations of the red cells preceding the formation of the black pigment.

This study was accomplished in 1883 by Marchiafava and Celli, who came to the conclusion reached by Marchiafava a few years previously that the red corpuscles do not give rise to the formation of pigment after their disintegration, but that the conversion of hæmoglobin into melanin occurs by degrees within the cells themselves.

These authors observed that the changes started by the red corpuscles and leading to melanæmia are ushered in by the appearance within the cells of spherical or ring-shaped bodies, easily stained by some of the aniline stains (methylene blue). Subsequent to this alteration small granules of pigment begin to appear in these little bodies,

which increase in size and number as the substance stainable by methylene blue increases.

These facts, obtained from a rich field of clinical material, enabled the authors to assert that the formation of pigment occurs within the red cells which have already undergone characteristic alterations, and this even before they had recognized the parasitic nature of the little bodies which could be stained by methylene blue. Naturally after the determination of facts such as these, the theories of Arnstein and of Kelsch, and *a fortiori* the hypothesis of Virchow and Frerichs regarding the splenic origin of the pigment, had to be abandoned.

The parasitic nature of the spherical and annular bodies being next established, the theory of melanæmia became at once elucidated. It is intimately connected with the life cycle of the parasite, so much so that a description of the genesis of the melanin cannot be separated from that of the parasite, for the study of which we refer the reader to the section on Parasitology.

In the same manner the distribution of the grains and blocks of melanin in the various vascular areas during an acute infection corresponds finally to the distribution of the parasites. This explains the fact that the distribution does not altogether correspond to that of inert powders injected into the blood, as Kelsch maintained, although as a rule it resembles it greatly. For instance, the melanosis of the brain in comatose pernicious fevers and the sometimes enormous accumulation of pigmented parasites in the intestinal capillaries in choleraic pernicious fevers are facts which it would be impossible to understand unless we bear in mind that we have to do, not with free circulating pigment granules, but with pigmented parasites. Consequently, the law of distribution of the black pigment proceeds from a study of the pathological anatomy of pernicious fevers, which will be found in the next section.

Formation of Melanin.—We will briefly outline the successive changes which occur in the formation by parasitic action within the red cells of melanin from hæmoglobin during an acute infection. We have seen that when fission of mature parasites is fairly accomplished, a residuum of segmentation is left, formed chiefly of a lump of melanin or an accumulation of black granules. When disaggregation of the spores has occurred, the pigment becomes free in the plasma, and thence is quickly taken up by the leucocytes and in part by the endothelium, especially in certain organs. The latter process is well seen in the brain and in the liver. This process occurs with every species of malarial parasite. But it is not only from the multiplying bodies that the pigment found within the leucocytes and the endothelium is derived. In part it comes from the parasite-infected red corpuscles

which die before the development of the parasite is complete, in part also from the pigmented parasites that may escape from the red corpuscles and so become free in the plasma. The first occurs chiefly in estivoautumnal fevers, in which, as we have seen, many red corpuscles become brassy; both brassy corpuscle and included pigmented parasite may be taken up by a leucocyte. The second is somewhat frequently noticed in the tertian, in which we may find free pigmented hyaline spherical bodies in the plasma, which are parasites or fragments of parasites that have come out of the red blood corpuscles. Finally, all the pigmented bodies which in man are sterile (forms of the anophelic cycle) end by becoming included when they cannot continue their regular development outside of the human body. The free or included pigment then accumulates in certain viscera, spleen, liver, and bone marrow, just as do inert powders injected into the circulation. But it is to be remarked that in these organs a large amount of pigment is formed *in situ*, or within their vascular areas, especially in estivoautumnal infections, because of the fact (upon which we have already laid emphasis) that it is precisely within the vessels of the spleen, etc., that the adult forms of the parasites accumulate and complete their development, during which process they form a notable amount of pigment. The alterations which in these viscera follow the parasitic invasion and the accumulation of melanin form a part of the pathological anatomy of acute and chronic malaria (see the following section).

Finally, in these same viscera in which it has accumulated, the black pigment is little by little transformed and destroyed, so that in a short time after the infection has ceased the melanosis entirely disappears. For a study of the succession of changes in the seat and character of the pigment in the melanotic viscera we refer the reader to the following section.

The question has been discussed whether all the black pigment which accumulates in the viscera, sometimes to an enormous amount, in persons who have had several attacks of fever is derived exclusively from the melanin elaborated by the parasite. For reasons which for brevity's sake we will not recapitulate (Bignami [16]) we have come to the conclusion that the melanosis of the viscera is chiefly the result of the melanæmia, that is to say, it is due to the deposit of the black pigment formed, during acute infection, in the circulating blood. In part it has a local origin, that is to say, it is derived from the slow transformation of the lumps of yellow pigment which are deposited or formed in the spleen and other organs from altered red corpuscles, which in grave infections die before the direct action of the parasite has transformed their hæmoglobin into melanin. As we know, this

transformation of the hæmosiderin into a black pigment which does not give the microchemical reactions of iron has been experimentally demonstrated by Schmidt (quoted by Neumann[17]).

Chemical Composition of Melanin.—Although the origin of melanin is known, we have but little knowledge of its chemical composition. Marchiafava and Celli noted the fact that even the finest of the black granules found within the red corpuscles and indicating the earliest stage of transformation of the hæmoglobin do not give a microchemical reaction of iron—and upon this point all the authorities agree. Carbone,[18] as a result of the chemical analysis of the pigment of a melanotic spleen, came to the conclusion that malarial melanin is for the greater part identical with hæmatin. This does not absolutely exclude the possibility of there being other pigments included with the hæmatin, although he maintains that this is not very probable. Such a chemical composition of melanin would be quite in harmony with what is known in regard to its origin. We know, in fact, that hæmatin is a product of the digestion, both gastric and pancreatic, of hæmoglobin, and it is therefore natural enough that the malarial parasite, when absorbing the hæmoglobin of the red corpuscles, should also give out hæmatin as a product of intracellular digestion. In other words, we may suppose that the parasite is nourished by the abstraction of the albuminoid constituents from the complex molecule of hæmoglobin, leaving the pigmented portion, that is to say, the hæmatin, unused. According to these researches then melanin is a transformation product of hæmoglobin, containing iron, but not in one of those combinations in which it is demonstrable by means of a microchemical reaction, as, for instance, the well-known reaction with potassium ferrocyanide and hydrochloric acid.

Is there Malaria without Melanæmia?—We have already said that the melanin, according to the researches of all recent observers, is found only in malarial infections. But the question may be raised, Is there malarial infection without melanæmia? Marchiafava and Celli,[15] as a result of their researches, inclined to the belief that this is the case, having seen cases in which the parasite accomplished all its life cycle up to fission without becoming pigmented. Many other writers, taking these observations as their basis, speak of a variety of malarial infection caused by a parasite of the red corpuscles which does not produce pigment (Mannaberg, Grassi, and Feletti). Still later, we began to doubt the existence of a form of malaria without melanæmia, having observed that even in cases in which an examination of the peripheral blood showed only non-pigmented parasites, and in the brain non-pigmented fission forms, in the spleen there were both pigmented parasites and pigment included in leucocytes.

It is certain that of late years, in spite of great richness of material, we have never seen a case of malaria without melanæmia. Yet we must consider the question as unsolved and leave the answer to future researches. The existence of an infection produced by parasites of the red corpuscles which complete their life cycle without producing pigment is, however, a well-established fact in the case of certain animals, the important instance being that of the so-called Texas fever of cattle. Dionisi has described an endoglobular parasite in bats which completes its whole cycle of existence without the production of pigment.

Yellow Pigmentation of the Viscera.—In addition to the black pigment we find in the viscera of malarial patients a variable amount of another pigment in the form of yellow or dark yellow granules or lumps; this is the ochraceous pigment the distribution of which has been studied by Kelsch and Kiener, Guarnieri, and Bignami. In contradistinction to melanin, this pigment gives the iron reaction with microchemical reagents, and is identical with the hæmosiderin of Neumann. As to its distribution, we know that it may be found in large amount in the liver and the spleen, in less amount in the bone marrow, and scantily in the kidneys.

In the liver this yellow pigment may be found included within the endothelium of the blood-vessels, but the larger part of it is in Kupfer's cells and the hepatic cells, differing in this from melanin which is never found in the epithelium of the liver. Frequently the pigmentation is most intense around the central vein, and shades off towards the periphery of the hepatic lobule. In some pernicious fevers, as Bignami noticed, this hæmosiderin pigmentation is so intense and so diffuse that it is more marked than the intravascular black pigmentation (melanin). Evidently all this hæmosiderin accumulated within the hepatic gland is destined to be used in the formation of the coloring matter of the bile.

In the spleen the yellow pigment is found within the globuliferous cells or free in the splenic pulp; in chronic tumors it is also seen, sometimes in large amount, within the vessels and splenic septa, being evidently deposited along the lymphatic tract. In the kidneys, in rare cases only, we see granules of hæmosiderin within the epithelium of the tubules, especially in that of the convoluted tubules.

The presence of this yellow pigment in the viscera of persons who have died of pernicious infection clearly indicates that not all the hæmoglobin of the destroyed red corpuscles is transformed into melanin by the action of the parasites, but that the acute infection determines the disintegration of a variable number of corpuscles by means of some other mechanism. We have already spoken of the

brassy bodies, which are the product of early necrosis of the red blood corpuscles, occurring while the parasites are still in process of development. In this alteration of the red corpuscles we see one source of the hæmosiderin deposited in the cells of the viscera, and perhaps it is the chief source during the acute infection. But we are unable to affirm that all the iron-containing pigment which is sometimes so abundantly present in the viscera of patients with chronic malaria and cachectics has the same origin. Above all, in grave post-malarial anæmic conditions we may find an abundant ochraceous pigmentation of the liver as in some cases of pernicious anæmia. Now, as we know that this anæmia may sometimes continue and even progress autonomously without relapses of fever or fresh parasitic invasions, we are forced to the conclusion that the pigment of hæmatic origin is in this case formed by the action of some hæmolytic substance as yet unknown. The genesis of the yellow pigment in these cases is naturally obscure, like that of the anæmia which goes on progressing after extinction of the infection, and in all probability it is due to a mechanism like that producing similar pigmentations in grave anæmic conditions.

Phagocytosis and the Changes in the Leucocytes.

From the days of the earliest researches into the blood of malarial patients, the attention of investigators has been attracted to the presence of leucocytes containing granules or blocks of pigment. We have seen above that the discovery of the malarial parasite was preceded by a series of researches, one result of which was to distinguish the pigmented leucocytes from other pigmented bodies differing from them, namely, the large pigmented parasitic bodies. With the increase in knowledge of the biology of the parasite came a corresponding increase in the comprehension of the phagocytic processes which occur in malarial blood, and the attention of many pathologists has been called to this matter because of the recent widespread interest in all that relates to the theory of phagocytosis.

Laveran held that the black pigment was taken up by the leucocytes after the disintegration of the parasites. Later Marchiafava and Celli established the fact that the white cells can take in not only pigment, but whole parasites and parasite-infected red corpuscles, and observed that the phenomenon of phagocytosis may also occur *in vitro* in ordinary preparations of blood, so that we may witness the struggle even under the microscope. They noted, moreover, that the vascular endothelium also plays an important part in phagocytosis in malaria. Metchnikoff later called attention to the importance of the part taken by the macrophagi in the spleen and liver.

Golgi, who studied phagocytosis in tertian and quartan fevers, discovered that the pigmented leucocytes are to be found in the blood in the early hours of every febrile attack, and concluded that phagocytosis occurs regularly in correspondence with determined phases in the life of the parasite. He attributed great importance to the phagocytic function of the leucocytes, holding that to it is due the spontaneous cure of malaria (as others also believed), and even went so far as to assert the probability that to this process is due the fact that not all malarial fevers become pernicious.

Similarly methodical investigations were pursued in the case of estivoautumnal fevers by Marchiafava and Bignami and by Bastianelli.

These researches chiefly concerned the circulating blood, but Guarnieri took up the question of phagocytosis in the liver, and Bignami extended his investigations to the various viscera in pernicious fevers. Both described the degenerative changes which occurred in the leucocytes and in the endothelium as a result of phagocytosis.

Let us briefly take up the questions which arise in regard to this process as it occurs during the course of malarial infection.

Elements which Act as Phagocytes.—These are (*a*) some but not all the varieties of leucocytes which are in the circulating blood; (*b*) the endothelial cells and the cells of Kupfer in the liver; (*c*) the cells of the splenic pulp, and (*d*) the large uninuclear leucocytes (without granulations) in the bone marrow.

(*a*) To the leucocytes in the circulating blood belongs the chief rôle in the phagocytosis which takes place during the febrile attack. We have stated that not every kind of leucocyte has a phagocytic function. Guarnieri noticed that the lymphocytes never contain black pigment, and this fact has been confirmed by Bignami and others, although Vincent claims to have seen small pigmented leucocytes in rare cases.

Nearly all observers agree that the most important agents in this process are the *large uninuclear leucocytes* (without granulations), and the so-called *transitional forms* (Uebergangsformen of Ehrlich and Einhorn). Next in order come the ordinary leucocytes with polymorphous nuclei and neutrophile granulations (multinuclear leucocytes); neither the lymphocytes nor the eosinophile white corpuscles perform phagocytic functions and it is altogether exceptional to find a pigmented eosinophile leucocyte. It therefore follows that the greater part of the phagocytes found in malarial blood belong to the group of macrophagi of Metchnikoff.

It is well known that the total number of leucocytes diminishes to below normal in ordinary malarial infections, while in pernicious

fevers it is increased (see the following section). But the fact is also worthy of note that the numerical proportion between the various kinds of leucocytes is more or less markedly modified; that is to say, there is an increase in the large uninuclear leucocytes and the transitional forms, and a diminution in the number of the polymorphous leucocytes, while the number of lymphocytes remains at about normal. This modification in the numerical relation between the large uninuclear leucocytes and those with polymorphous nuclei is found in the ordinary infections—tertian, quartan, estival, as well as in the pernicious forms; in the last named, however, the increase of the large uninuclear cells chiefly attracts attention, while it is the least marked in the first attacks of a primary infection.

All observers who have examined the blood in pernicious fever are surprised by the large number of macrophagi found in some cases. And, indeed, the increase in number of the large uninuclear leucocytes is much more noticeable in grave malarial infections than in those conditions, such as grave anæmia and hyponutrition of the organism, in which, as Ehrlich has shown, the same thing occurs. We take the following figures from the work of Bastianelli:

Primary infection, fifth day of the disease, fatal perniciosa: Lymphocytes, 18.1 per cent.; uninuclear large cells, 25.3 per cent.; transitional forms, 6.1 per cent.; multinuclear neutrophile cells, 50.2 per cent.; eosinophile cells, 0.4 per cent.

Relapse, fatal comatose perniciosa: Lymphocytes, 19.1 per cent.; large uninuclear cells, 34.2 per cent.; transitional forms, 6.8 per cent.; multinuclear cells, 39 per cent.; eosinophile cells, 0.6 per cent.

The numerical increase in the uninuclear cells in relation to the decrease of the multinuclear ones does not normally attain the large proportions found in the two cases given above, but it may be considered as a constant occurrence in all the malarial fevers. Vincent and Billings confirm these facts. We must therefore conclude that in malarial blood there is an increase of precisely those elements which play the chief part in phagocytosis, namely, the large uninuclear cells. These bodies enter into the circulation in a larger number than normal, evidently by a process of chemotaxis; or in other words, for the same reason that in other morbid conditions multinuclear leucocytes are poured into the blood. The chemotaxis is specific, and is exercised by determined substances upon a particular species of leucocyte; in fact, if in an individual in whom malaria alone has determined a percentage increase in the large uninuclear cells, there occurs some inflammatory process, such as pneumonia or erysipelas, the multinuclear cells in their turn increase in the blood

(inflammatory leucocytosis) under the influence of the specific stimulus of the streptococcus or of Fränkel's diplococcus.

(*b*) The pigmented endothelial cells are found circulating in the blood only in the course of the gravest infections; they evidently become detached from the vascular walls as a result of the alterations which they undergo after the inclusion of the foreign bodies which they take up. An anatomico-pathological study of the viscera can alone give us an exact idea of the extent to which this function of the endothelium is exercised. In some cases the number of pigmented endothelial cells in the brain is remarkable. It is evident that the accumulation of adult parasites in the cerebral vessels and the resulting relative slowness of the circulation favor the development of this function. But while this function of the endothelium helps to free the blood-vessels from injurious matters, the degenerative changes which follow phagocytosis cause on the other hand lesions of the vascular walls which increase the difficulty of the capillary circulation, and contribute still further to its retardation.

Even in the liver the endothelial cells are actively phagocytic; indeed, as Bignami has noted, after the active infection has ceased the pigmentation of the endothelium persists longer than does the presence within the capillaries of melaniferous leucocytes.

Guarnieri has noticed the same thing in the case of Kupfer's cells; he found in them not only black pigment, but grains and clumps of hæmosiderin (yellow pigment) in variable amount—to a very large amount in some cases of pernicious fever.

(*c*) The cells of the splenic pulp take an active part in phagocytosis in all acute malarial infections. This is demonstrated not only by anatomico-pathological studies in pernicious fevers, but also by the examination of the splenic contents extracted by puncture in cases of ordinary fever. The number of pigmented globuliferous and parasite-containing macrophagi is truly enormous in grave infections; but no included bodies are ever found in the lymphocytes of the Malpighian follicles. The blood of the splenic vein is rich in pigmented macrophagi or the débris of red blood corpuscles, for which reason we are led to think that a large number of similar elements which accumulate in the capillary network of the liver are derived from the splenic pulp.

(*d*) Phagocytes of similar histological character are also found in the majority of pernicious fevers in the bone marrow, especially of the spongy bones, not only within, but also outside of the blood-vessels. Certainly some of these elements are derived from the blood which deposits them in the large medullary veins, but everything points to the belief that in large part they are medullary cells which have exercised a phagocytic function *in situ*. It is well known that

the large uninuclear cells without granulations (the chief elements in malarial phagocytosis) originate in this way in the spleen and in the bone marrow, and, according to Ehrlich and Lazarus, chiefly in the bone marrow.

From the preceding statements it is evident that phagocytosis occurs in the whole vascular system during acute malarial infections, but chiefly in certain viscera, and precisely in those in which, as experimental pathology teaches us, are deposited the corpuscular extraneous substances injected into the circulation, namely, the spleen, liver, and bone marrow. After the cessation of the acute infection the phagocytes which have gathered up pigment or parasites elsewhere are also deposited in the organs, which participate actively in the process by means of their own cells (cells of the splenic pulp, large uninuclear cells without granulations of the bone marrow).

The substances which may become included in the cells are varied in nature. In the order of frequency come first the clumps of pigment and the residua of segmentation which remain free after the multiplication of the parasites; less often we find complete fission forms, either free or within erythrocytes, or isolated spores. This occurs in tertian and quartan as well as in estival fevers. As to the young forms and those in process of development, it is to be noted that only in summer-autumn fevers do they become included in phagocytes to any marked extent; the reason for this is found in the early alterations undergone by the parasite-infected red blood corpuscles in this group of fevers. In fact we find included, in the order of frequency, parasites with central pigment or peripheral granules of pigment, or even non-pigmented young forms contained in brassy or pale corpuscles; very rarely, parasites in various stages of development contained in corpuscles in which the methods of examination at our command do not permit with any certainty of the recognition of any alteration. We have already noted the fact that even in estival fevers the parasites bring about certain changes in the physical properties of the red blood corpuscles—such, for example, as a certain viscidity of their surface—which in all probability favor the appearance of the phagocytic function.

In tertian fever, on the other hand, it is rare to find young parasites within the leucocytes. In the spleen of tertian-fever patients, Bastianelli and Bignami have seen included in the leucocytes the pigmented hyaline spherical bodies which originate from parasites that have come out of red blood corpuscles and have become disintegrated in the plasma.

All these data lead us to the conclusion that phagocytes may take in not only the adult and actively multiplying bodies, which in the

process of multiplication leave the red blood cell and become free in the plasma, but also forms in process of development and young bodies, whenever the red corpuscles which contain them undergo some early and profound change which causes them to behave towards the phagocytes like foreign bodies in the blood current. As this early necrosis of the red corpuscles occurs with regularity in estival fevers and only exceptionally in the others, we can readily understand why the phagocytosis of the parasite-infected red corpuscles is of great importance only in the group of estival fevers.

In addition to these bodies, the phagocytes may take up the adult forms incapable of multiplying in the human organism—the gametes. When the latter remain in the host they go on to those degenerations which were recognized before anything was known about their subsequent development in the intestine of the mosquito.

Finally, in phagocytes, especially such as are situated in the viscera, we find a variable quantity of fragments of red corpuscles which by further transformation of the hæmoglobin give rise to grains or clumps of hæmosiderin. In some pernicious fevers this occurs extensively in the spleen and liver, especially in the endothelium and in Kupfer's cells.

To sum up then, we find that phagocytosis acts (*a*) on substances which originate from the parasites, that is to say, the black pigment and the residua of segmentation; (*b*) on the parasites themselves when they become free in the plasma, or when they are contained within much altered red cells, and (*c*) on débris of red corpuscles and entire dead corpuscles.

The phenomenon is evidently the result of a chemotactic action between the phagocytes and the substances which they take up, an action which is developed only under the conditions mentioned. The parasite-infected red corpuscles which have not been specially changed escape phagocytosis.

The included substances become modified in various ways. The hæmoglobin of the amœbiferous red corpuscles goes through the changes which have been described until the latter are mere shapeless rusty masses. The melanin, which in the leucocytes of the peripheral blood is found in grains, or needles, or distinct blocks, becomes gathered into large formless masses. These are usually found in the phagocytes of the spleen and liver, while in the circulating blood they are seen only in grave infections, chiefly in pernicious fevers. As to the included parasites, they remain clearly recognizable and capable of staining so long as the red blood corpuscles with which they have been absorbed into the phagocytes persist. The free bodies apparently change and disintegrate very rapidly after their

inclusion, so that it is difficult to recognize them; only the fission forms, the bodies with blocks of central pigment, and also the free spores maintain for a length of time, as yet undetermined, their normal capacity for staining. Yet if not so very long after the cessation of an acute infection a spleen be examined with even the best coloring matters, we shall not be able to recognize parasitic bodies within the leucocytes.

This leads us to think that all the parasites included in white corpuscles are destroyed with more or less rapidity and become incapable of further development. Only as to the spores, which remain recognizable within the leucocytes longer than do the other parasitic bodies, Bignami advances the theory that they may persist alive and take on a new development after necrosis of the white cells which contain them. He also further supposes that the said spores, which are naked in the beginning, may later become surrounded by a membrane and thus, when still within the leucocyte, escape our methods of coloration and demonstration. Those hypotheses which were advanced to explain the period of latency of the infection cannot at the present time be refuted, although, to tell the truth, there are no new facts to sustain them.

We may, however, positively exclude the idea held by Golgi and supported by Monti that the estivoautumnal parasites are capable of continuing their development within the leucocytes or the tissue cells. The chief argument in support of this theory is the fact that within the phagocytes may be found every phase of the parasite from the youngest to that of sporulation; yet, as we have already stated, the number of young parasites included is small in comparison with the number of bodies with central pigment or in fission, so that it will not do to assume that the adult bodies come from parasites which have been included in leucocytes from the earliest stage of their existence. On the other hand, direct observation shows that most of the parasites become included only in the later stages of their life cycle which has developed normally within red blood corpuscles. We would add also that all the arguments derived from probability and analogy oppose this hypothesis.

Degenerative Changes in the Phagocytes.—In the leucocytes, as in the other cells that have acted as phagocytes, degenerative changes frequently occur which may go on to necrosis. These alterations may be seen in the leucocytes which are found in the peripheral blood only in grave infections and in pernicious fevers; as a rule, however, they are seen also in the spleen, liver, and bone marrow, where the degenerating and necrotic elements are indeed often present in great abundance. The principal alterations are:

(*a*) *Fatty degeneration*, which chiefly attacks the large uninuclear leucocytes usually after they have taken in a large number of foreign bodies; they then appear as large cells, even two, three, or more times the normal size of an ordinary leucocyte, which contain in their protoplasm a large number of spherical shining bodies of various sizes, which in fresh preparations are sometimes oscillating, which disappear in dried preparations, are not stained by aniline colors, and are not visible in sections fixed in alcohol. A similar degeneration may be seen, although rarely, in leucocytes which do not contain other bodies. Bignami found the same change, in some cases of acute malarial infection, in the endothelial cells of the spleen and the liver, and in Kupfer's cells. For the most part these little spherical droplets are of a yellowish color; sometimes the yellowish coloration is seen throughout the whole cell, suggesting a slight imbibition of hæmoglobin such as has been noted to a greater or less extent in the leucocytes in certain cases of intoxication by hæmolytic poisons such as pyrodin. This special form of alteration may be studied in preparations fixed in osmic acid; in them we note that the granules or droplets scattered in the protoplasm have generally only their outlines darkened. This fact makes it difficult to decide what is their exact nature, although their other properties, such as solubility in alcohol and ether, would make it appear that they are fat droplets.

(*b*) *Vacuolization of the protoplasm*, like fatty degeneration, chiefly attacks the macrophagi; in this case also the cell is swollen and its protoplasm appears to be rarefied by the presence of numerous vacuoles in some of which we may see included bodies. This alteration may also be seen in the circulating blood, especially in grave cases of estival fever. In the leucocytes with vacuoles as in those with fatty degeneration, the nucleus in the stained preparations often remains of normal appearance; in other cases it shows retrogressive changes, which will be described presently.

In fresh preparations, however, we often do not see it at all, and when examining a fresh specimen at a temperature above 30° C. (85° F.), we are surprised to see these protoplasmic masses of occasionally enormous size, filled with vacuoles or shining droplets, and without perceptible nuclei, sometimes presenting active amœboid movements.

(*c*) *Nuclear changes* in the phagocytes are well seen in preparations fixed in absolute alcohol and ether and stained with hæmatoxylin and eosin. Sometimes the nucleus is fragmented in irregular masses which become intensely stained in a somewhat uniform manner (*nuclear fragmentation and chromatolysis*). At other times only the membrane of the nucleus is recognizable, the nuclear chromatin having disappeared, and the whole nucleus is but faintly stained. From this

change, a series of transitional stages lead up to the phagocytes which present all the signs of necrosed elements (coagulation necrosis); they are formless masses of protoplasm, with irregular outlines, without trace of nucleus, staining faintly, and sometimes dotted with irregular small bodies that are stained by hæmatoxylin and represent residua of the nuclear chromatin. In preparations from the spleen we also see protoplasmic masses of varying size, without recognizable cellular structure, which, as everything leads us to believe, originate in the fragmentation of the necrotic phagocytes.

Similar changes may be seen in the endothelial cells. While many phagocytes undergo these retrogressive changes which partly account for the leucopenia observed during the febrile attack of malaria, other cells multiply actively in the hæmatopoietic organs. Multiplication by karyokinesis of the endothelia and Kupfer cells has been seen in the liver by Guarnieri and by Bignami; the latter further described the multiplication by karyokinesis of the cells of the splenic pulp and of the large uninuclear leucocytes of the bone marrow. The new elements replace those which have become necrosed from the exercise of their phagocytic function. It is to be noted that the cells in karyokinesis are as a rule non-pigmented. In pernicious infections karyokinesis of leucocytes may occur even in the circulating blood, as has been observed by Bastianelli as well as ourselves. The process is absolutely similar to that seen in the splenic pulp and in the bone marrow. In some pernicious fevers these forms are somewhat numerous, two or three being found in one preparation, but they are always less in number than in preparations from the splenic pulp. By this active proliferation the hæmatopoietic organs provide an abundant supply of new large uninuclear leucocytes, of those elements which are of chief importance in malarial phagocytosis. And as in grave anæmic conditions erythroblasts in mitosis may issue from the hæmatopoietic organs, a similar thing may happen with the white corpuscles in malaria; that is to say, immature forms of these elements may be poured out into the blood, the loss of these being greater in consequence of the infectious process.

The development of the phagocytic function is in intimate relation to the life cycle of the parasites and to the evolution of the fever. This relationship in the case of tertian and quartan fevers has been established by the researches of Golgi, which we are able to confirm by the results of our personal observations. Golgi sums up his observations as follows: "We shall look in vain in the circulating blood for manifestations of phagocytosis in regard to the malarial parasites when the latter are in their endoglobular stage, and even when they are found in the phase preceding their perfect maturation; on the

other hand we can readily perceive the phenomena of phagocytosis when the parasites have reached maturity and are about to become segmented or have already divided. They begin with the onset of the attack, are most evident from three to four hours later, and terminate a few hours after the end of the attack, but even later there are events which seem to represent the continuation of the process. The phenomena, in their entirety, occur in a period of from six to eight or twelve hours." The author describes as existent in the blood, during the period of development of the attack, white corpuscles containing bodies in process of segmentation or with well-formed spores, or isolated masses of pigment. Later there are in the blood leucocytes containing the same malarial bodies in a more advanced state of disaggregation. After ten or twelve hours the destruction of matter is accomplished, and the phagocytes disappear to undergo the same changes and evolution in the next attack.

From these facts Golgi formulates the following law: "Phagocytosis is a process which develops periodically like a regular function of the white cells, a function which develops in a certain manner corresponding to determined phases in the evolutionary cycle of the malarial parasite and in a certain period of each febrile attack."

From this standpoint there are certain differences between the quartan, the tertian, and the estival fevers upon which we shall later dwell more at length. Before that we wish to describe the results of our observations in this group of fevers which were described by us in 1892. From what was stated at the beginning of this chapter it is evident that phagocytosis occurs throughout the vascular system, but preferably in certain viscera (spleen, liver, etc.), for which reason what is seen in an examination of blood from the finger should be regarded as a mere episode in a process which, at least in this group of fevers, is chiefly carried on in the internal viscera, so that if we do not find examples of phagocytosis in the peripheral blood we are unable to state that the process is not taking place elsewhere.

In summer tertian fever the phagocytic bodies, especially the leucocytes including the round masses of pigment found at the centre of fission forms, begin to appear at the onset of the attack, and during the attack they usually increase in number until towards its close they become exceedingly numerous. In typical cases of estival tertian the largest number of pigmented white blood cells is usually seen at about the time of the precritical elevation of temperature. During the brief period of apyrexia the phagocytes diminish to a notable extent, and in rare cases they disappear, to be seen in greater number at the beginning of the next attack.

These occurrences can be verified almost without exception; in-

deed, there are some cases of mild estival tertian fever in which for a short while no parasites can be found in the peripheral blood, but in which a diagnosis of malaria can be made from the presence of pigmented leucocytes.

This cyclic function of the white corpuscles, which is accomplished in correspondence to the febrile attacks, may be easily followed in cases of infection of recent date; but it is not seen in cases in which the malarial infection has lasted for some time. In these cases leucocytes are always found, and it is not always possible to tell when they increase or diminish—the difficulty of decision is increased by the brevity of the periods of apyrexia.

That in these cases phagocytes should be found in the blood not only during and shortly after the febrile attack, but also during the whole period of apyrexia may be easily explained. It is well known that when the acute infection ceases, the phagocytes slowly leave the general vascular system of the viscera, lungs, intestines, kidneys, etc., and collect in the spleen, liver, and bone marrow. Now experience has shown that this purification of the circulation takes for its completion many hours and in some cases several days, according to the gravity of the parasitic invasion; we can therefore understand that during the period of apyrexia interposed between two attacks we should continue to see phagocytes circulating in the blood, when because of a succession of febrile attacks numbers of them have polluted the capillary system of the viscera. For the same reason we can see why in the apyrexia between a series of relapses the pigmented leucocytes may be found in scanty number in the blood for several days—five or six after the parasites have completely disappeared.

In the majority of cases of pernicious fever the phagocytes are usually exceedingly numerous. We are more liable to find in these grave infections than in the ordinary estival tertian, in addition to the pigmented leucocytes, phagocytes containing complete sporulations, and parasite-infected brassy bodies, pigmented and globuliferous endothelial cells, etc. The most striking thing in these cases is the presence of the large macrophagi which have already been described, and some of which show signs of degeneration. A cure having been effected by means of quinine, we usually continue to see phagocytes circulating in the blood for five, six, or eight days.

After the fever has been cured by quinine, the pigmented leucocytes are seen in the circulation for a variable time, even for days, if the parasites and phagocytes were numerous in the blood before the use of the remedy. During the action of the quinine, we often are able to perceive an increase in the number of the pigmented and

globuliferous leucocytes—the reason for which is found in the fact that the phagocytes seize all the parasitic bodies the development of which has been arrested by the quinine; and that, moreover, as we have found, the transformation of the red corpuscles into brassy bodies is favored by the remedy itself—whence there is an increase in the amount of material which the phagocytes might seize. This increase in phagocytosis after the administration of quinine is seen more perfectly in pernicious fevers than in ordinary infections, and, what is worthy of note, not only in pernicious fevers which are destined to recovery, but in those which have a fatal ending.

In cases in which a spontaneous cure has occurred, a careful examination of the blood has not given us constant results. Sometimes when the attacks have become weaker and finally disappeared we have seen an increase in the number of phagocytes as compared with that seen in the days preceding those in which the infection tended to become spent; in other cases, on the contrary, the diminution of pigmented leucocytes appeared to keep pace with the disappearance of the parasites.

As was noted by Bignami and Bastianelli, when crescent bodies persist in the blood after cessation of the fever, we continue to see pigmented leucocytes at intervals, just so long as the crescent bodies are present; not infrequently we also see included round bodies with pigment in wreath form, or true crescents. It is to be noted that in these cases the pigment contained within the leucocytes is in fine needles or rods, so that we can recognize it as having come from degenerated and "included" crescents.

If we compare these facts with those observed in quartan and tertian fevers, we note that even in estivoautumnal fevers the most intense phagocytosis corresponds to the period in which the parasites are multiplying. But there are some differences, which are indicated indeed by what has been said above. In the first place, in grave fevers we often find globuliferous cells, and especially macrophagi, containing parasite-infected brassy bodies. The included red corpuscles may be entirely decolorized, or may appear almost normal; they may contain young non-pigmented plasmodia, or pigmented forms, or small bodies with central pigment in fission, or bodies of the crescent stage. Therefore, in grave fevers, the process of phagocytosis may occur even when the parasites are endoglobular and when they are in the phase preceding maturity.

Everything tends to show that the special modifications which the parasite-infected red corpuscles may undergo in this kind of fever partly explain their inclusion within the leucocytes, an inclusion which may occur even when much of the parasite-infected glob-

ule survives. These facts, as we have said, are not observed in the corresponding stages of the development of tertian and quartan parasites.

Moreover, in grave fevers, we note with less distinctness than in quartan and tertian the periodicity of phagocytosis. The reason for this difference is found in the shortness of the periods of apyrexia in estival fevers, the fact that in these fevers the multiplication of the parasites from which is derived the greater part of the substances taken up by the phagocytes occurs chiefly, if not exclusively, in the internal organs, and finally in the greater tendency of these fevers towards an irregular course.

Importance of Phagocytosis.—The facts so far stated demonstrate that phagocytosis is an important process in malarial infection. But if we attempt to ascertain what part phagocytosis takes in the defence of the organism against the parasitic invasion, what influence it exercises upon the course of the infection, and if to it is due spontaneous recovery, we come up against many difficulties in the interpretation and comprehension of the facts. For this reason it is not to be wondered at that the reports of the various investigators do not agree. They are to a great extent influenced in their reasoning by the theory of phagocytosis, to which so many attribute the chief part in the defence of the organism and in immunity, while others accord it a secondary place.

Upon one point there seems to be no possibility of doubt, and that is the function which belongs to the phagocytes of clearing the vascular system from the detritus and dead corpuscles deposited during acute infections. When we think of the large amount of black pigment that is set free in all parts of the vascular system in some pernicious fevers, and of the great number of degenerated red corpuscles and of free parasites which become included in endothelial cells and in leucocytes, and recall the fact that all these matters are deposited in certain viscera, the spleen and liver chiefly, in the course of a few days, we are able to realize the importance of the function. In a relatively short space of time the vascular system of the most important viscera, as for instance the brain, is restored to conditions essential to a normal circulation of the blood. We may add that the return to a normal condition of the spleen, liver, and bone marrow in which the débris of the infection is deposited and remains for a while, is in part the result of a series of phagocytic processes, which are accomplished slowly and in regular succession, as we may see in the study of the pathological anatomy of chronic malaria. In the course of acute infections there occur in certain organs degenerative changes in the cells of the parenchyma, and even more or less

extensive actual necrosis of the tissue—this has been observed chiefly in the spleen and liver. Now these necrosed zones are eliminated and replaced by a tissue of neoformation which originates in the special cells of the organ itself only when the whole vascular system of the necrotic area has been freed and cleansed from the foreign bodies deposited therein. This has been demonstrated by Bignami in the liver, in which the process can be clearly seen.

Of the organs mentioned above, the bone marrow is the one which most rapidly becomes cleansed of the pigment and of the foreign elements deposited there during an acute infection. In fact, as Bignami noted in sections from cadavers of persons dying at varying periods after the cessation of the infection, we find that the marrow contains a scanty amount of black pigment, or scarcely any in cases in which the melanosis of the spleen and liver is still intense. Now this return to the normal, which cannot help having a beneficial action upon the activity of the bone marrow as a hæmatopoietic organ, is ultimately the work of the phagocytes. Wherefore, even limiting the function of the phagocytes to that of "spodoferous" and "spodophagous" cells (to use an expression adopted by us elsewhere) we find their importance to be great by reason of the restitutio ad integrum of the tissues effected through their agency.

Relation of Phagocytosis to the Intensity of Infection.—What influence has this function in determining the gravity or lightness of the disease? Or, in other words, are pernicious infections such because of deficient phagocytosis, and the mild infections mild by reason of the energy of this process? Golgi, in his researches into quartan and tertian fevers, was led to attribute an importance to phagocytosis from this standpoint which we are unable to admit. He noticed that during each febrile attack the leucocytes contained not only the retrogression products of the parasites, but also a certain number of parasites themselves. If this were not the case, and if all the parasites invariably completed their life cycle, then, according to this writer, "every case of intermittent malarial fever would go on increasing in severity even to the point of transformation into pernicious fever," which, as we all know, is not the case. Now from our observations we cannot admit that phagocytosis is the only factor in preventing the aggravation of all cases of fever, and especially the conversion of quartan and tertian into pernicious forms. In fact, from the time that the parasites of quartan and tertian infections have been known, there have been no examples of pernicious fever caused by them. This, as we have observed elsewhere, leads us to attribute this constant fact to the biological properties of this group of parasites, and not to functions of defence and individual reactions which are so apt

to be variable in their action. Pernicious fevers are caused only by parasites of the summer-autumn variety, and are due especially to the virulence and toxicity of the parasites in this group.

Even limiting the discussion to grave infections, we cannot admit that some of them become fatal from insufficient phagocytosis and others are cured because of the efficacy of this defensive process. A close examination of the facts will show us that there are fatal pernicious fevers with extensive phagocytosis and others in which the process is feeble; the first occur usually in relapses of malaria, the second in primary affections. As a rule, however, phagocytosis is very active in grave infections, so much so that up to a certain point we may consider the number of phagocytes to be in proportion to the number of parasites; in other words, phagocytosis is most active when there is the greatest number of parasitic forms in the condition necessary to admit of their being taken up and "included," as we endeavored to demonstrate above. This condition of things leads us to believe that the result of these infective diseases is in part dependent upon the primary number of parasites, and is in part under the control of a complex series of factors which in their entirety constitute what is known as the power of resistance of the organism. According to our way of thinking, to explain the resistance and relative immunity acquired by malarial patients during the course of an infection by phagocytosis alone, is to simplify unjustifiably a very complex process. Discussion has also arisen over the part played by phagocytosis in spontaneous recovery from the fever, and in recovery due to the action of quinine. We will take up this question in the section on Treatment.

Malarial Anæmia.

Clinically and from examination of the blood we find that after every febrile disease of a certain gravity and duration there is a diminution in the number of the red blood corpuscles; but in no other infection is anæmia produced with the same rapidity and to the same extent as in malaria. This fact was well known to physicians for some time before the discovery of the malarial parasite, and was clearly demonstrated by the researches of Kelsch, made in 1875–76 according to the method of Malassez. This investigator determined the amount of the loss of the red blood cells in the primary attacks, in relapses, and in malarial cachexia. He ascertained the differences which exist from this standpoint between simple and pernicious fevers, and then he studied the behavior of the leucocytes. At a later date, after the discovery of the parasite, Dionisi endeavored to ascertain what relation exists between the variations

in the red corpuscles and the number and variety of the parasites. Dionisi and Poletti further studied the reconstruction of the blood. If we add to these the researches of Rossoni upon the variations in the hæmoglobin, those of Viola upon the density of the blood and the isotonia of the red blood cells, etc., we can see that of all the forms of anæmia the malarial is one of the best known and understood.

In the first place, let us ascertain the behavior of the red blood cells in acute malaria, distinguishing the simple from the pernicious fevers.

Even in acute malaria, in spite of the absence of grave symptoms, the febrile attack exercises a rapid and visible influence upon the number of red blood corpuscles. Thus a patient of vigorous constitution in the first four days of a quotidian fever, or a remittent fever of first invasion, may show a reduction to 2,000,000 red blood corpuscles, or there may even be a reduction of 1,000,000 per cubic millimetre within twenty-four hours. Twenty or thirty days of simple quotidian or tertian fever suffice to lower the number of red blood cells from 5,000,000 per cubic millimetre to 1,000,000 and even less (Kelsch).

It is important to note that when after a certain number of attacks the patient falls into a moderate condition of anæmia the loss of red cells in the successive febrile attacks is much less than in the beginning. For this reason, according to Kelsch, we may during the course of a malarial infection distinguish several periods, during which, as a rule, the intensity of the anæmia produced by single attacks becomes progressively less. Thus, for instance, in a robust individual who is attacked by estivoautumnal fever, we usually observe a first period in which the febrile attacks are severe and the symptoms grave, and this is characterized by a rapid diminution of the red blood corpuscles; a second period follows, in which the attacks are still severe, but have less tendency to become continuous, and in which the diminution of red blood cells continues, but is less marked than at first; and finally there is a period distinguished by groups of isolated attacks with more or less lengthened periods of apyrexia, in which the number of red blood cells does not markedly diminish, but remains at a certain minimum point, the loss during the attacks being compensated for during the apyrexia.

As an example of the great amount of the initial loss in estival infection, we recall a case in which, three days after the onset of a summer-autumn fever, Dionisi found the number of red blood cells reduced to 2,625,000; in another case, only twelve hours after the onset of the attack, the number had fallen from 4,500,000 to 4,000,000.

In pernicious infections the diminution is usually more rapid and

more marked. But these infections may also be divided into two groups according to whether they occur at the beginning of the disease or in patients already anæmic from the result of previous infections. In the first class of cases, the diminution may be most rapid and most marked, so that, according to Kelsch, in a robust individual in the course of one day only, the number may go from normal to 1,000,000 per cubic millimetre; in the second class of cases the diminution is not usually more than 1,000,000 to 2,000,000 red blood corpuscles in the same period of time.

It is to be noted that this anæmia is produced even in the cases of pernicious fever which are not accompanied by elevations of temperature—the so-called larval or masked pernicious fevers. These data, observed by Kelsch, have been confirmed in all essentials by subsequent researches.

Dionisi has endeavored to ascertain what relation exists between the variation in the number of the red cells and the number and species of the malarial parasites found in the blood. He found in the first place that in estivoautumnal fevers and in quartan and ordinary tertian as well, the reduction of the red corpuscles does bear a relation to the number of parasites in fevers of first invasion. In the relapses, on the other hand, even when there are many parasites, the diminution of red cells occurring after an attack is much less than in a fever of first invasion under the same conditions. Indeed, although a series of attacks may have caused a marked reduction in the number of red blood cells, a fresh febrile attack may not cause any further reduction—in fact, in spite of the attack there may even be an increase in the number of red corpuscles.

Of prime importance is the fact that when crescents alone are in the blood there is no anæmia produced, nor do the crescents interfere with the reconstruction of the blood, if that has begun.

The *leucocytes* also are subject to constant and important numerical variation in acute infective processes. As a result of the febrile attack, they diminish with constancy, not only absolutely, but also relatively to the red corpuscles; according to Kelsch, their numerical diminution is even more rapid and proportionately greater than that of the red cells. Indeed, in some observations made by this writer we find the following proportions of red to while cells: 1,418: 1; 1,243:1; 1,003:1; at the third attack of a tertian 3,365:1; in a cachectic 2,377:1.

The absolute and relative diminution of the leucocytes is especially marked in cachectics and in the profoundly anæmic, in whom it tends to become permanent. In Kelsch's observations the few exceptions to this rule were three or four cases of profound malarial

anæmia, in which, during a condition of great gravity, with prostration, drowsiness, etc., the writer found an increase in the white relatively to the red cells, the figures being 1 per 192, 118, and 112 respectively.

Concerning the number of the leucocytes during and after a febrile attack, Kelsch found that the diminution in their number is not only marked, but also rapid and continuous; that is to say, from the first hours of the attack the leucocytes became reduced uninterruptedly until we find only a half or even a third of the number existing previous to the attack. This over, the number increases much more slowly than it decreased, fifteen to twenty hours and even one to two days elapsing before the physiological balance between the red and the white corpuscles is restored. In some cases in which Kelsch was able to count the corpuscles at the beginning of the attack, it appeared to him that there was a slight and very transitory increase in the number of leucocytes. This occurrence, which is the opposite of what takes place during an advanced attack and after it, has recently been observed by Vincent also, who says that the number of white cells is suddenly raised during the initial chill, while it is lowered to a sometimes considerable amount in the hot stage and at the end of the attack. He adds, however, that this increase is not always to be found, that it is sometimes very slight and sometimes altogether absent. This irregularity accounts for the reserve with which Kelsch expresses himself upon this point.

With this *leucopenia* observed in simple malarial infections is in strong contrast, according to Kelsch's observations, the relative increase in the leucocytes found in pernicious infections. In these fevers he has found at times 10,000, 20,000, and even 35,000 leucocytes per cubic millimetre. For instance, in a case of comatose pernicious fever there were 15,353 white cells, in an algid perniciosa, 20,445. This increase comes to a rapid end after recovery from the pernicious attack. For instance, in a case of comatose pernicious fever which began during the night, Kelsch found on the following morning (temp. 39° C.—102.2° F.) 21,873 white to 3,024,320 red cells—a proportion of 1:138; in the evening, during the same attack (temp. 37.5° C.—99.6° F.) there were 11,750 white to 2,820,000 red cells—a proportion of 1:240; and after recovery from the attack, 3,796 white to 1,998,440 red cells—a proportion of 1:326.

If pernicious fever attacks a chronic or cachectic malarial patient, in whom, as a rule, the number of leucocytes is much less than the physiological amount, the number of white cells tends temporarily to rise to normal proportions.

Laws Governing the Reconstitution of the Blood.—Leaving until

later an inquiry into the pathogenesis of this malarial leucopenia, and the reasons why simple fevers behave differently in this respect from the pernicious fevers, let us see what are the laws which govern the reconstruction of the blood. In the first place we must remember that the time necessary for the blood repair varies in different individuals according to their age, vigor, etc. Moreover, in addition to these individual differences which may be marked, there are others relating to the nature of the infection and to its duration—indeed, the injuries produced in the organism by a long series of relapses exercise a great influence upon the activity of the reparative processes.

A relatively rapid reparation occurs in mild quartan and tertian infections of somewhat recent date (Dionisi, Poletti). Thus in some cases of double tertian in which the red corpuscles had decreased in number to a minimum of 2,500,000 in one case, and much less in the others, the complete integrity of the blood was restored under the influence of treatment in a month or a little over (Poletti). It should be noted, however, that even in these cases in which the conditions were most favorable for the reconstitution of the blood, the return to normal occurred more slowly than in anæmia following blood-letting or the action of certain blood poisons, such as pyrodin.

In the estivoautumnal infections the restoration of the blood occurs even more slowly than in the quartan and the tertian; in some cases, in the first days of apyrexia there is merely an attempt at repair, which may be followed by an eight to fifteen days' period of diminution or of a stationary condition. The following are some of Dionisi's statistics: In a case of relapsed estivoautumnal fever in which the number of red blood corpuscles had fallen to 2,300,000, after eight days of apyrexia and well-being, under treatment with iron, there were only 2,370,000 red corpuscles per cubic millimetre. In another case of relapsed fever, in which the number of red cells was diminished to 3,000,000, the result after eight days of apyrexia, well-being, and tonic treatment was an increase to 3,650,000. In one case of primary estival infection, in which the number had decreased to 3,270,000, it remained about the same for the first five days of apyrexia, and then, in spite of the administration of iron, again diminished during the six following days to 2,370,000 per cubic millimetre. But, as we have already stated, even in estivoautumnal fevers we find marked individual differences. Thus, for example, in a robust person of thirty-five years, after four days of estivoautumnal fever, the red corpuscles decreased to about 2,000,000 per cubic millimetre; in the subsequent eight days of apyrexia, the patient taking quinine, they gradually and progressively increased up to 4,340,000.

In hemorrhages, as we know, and the same thing occurs in pyrodin

poisoning, the reparation of the blood usually occurs with some rapidity at first and then more slowly. We have already seen that this is not always so in malarial patients. In some cases, it is true, there is so rapid an increase of the red corpuscles in the first days of apyrexia as actually to suggest the idea that it is not really a process of repair, but a relative increase of the red cells in proportion to the plasma; but more frequently perhaps, the increase is apt to be somewhat slow at first and then more rapid. In one case of double tertian, after a series of five attacks the red cells were reduced to about 2,000,000 per cubic millimetre; in the first thirteen days of apyrexia, the patient being under treatment with iron and arsenic, the red cells rose only to 2,640,000; two days later there was a rapid increase up to 3,860,000, and after four days more the red cells were found to the number of 4,000,000 (Dionisi). These numbers suggest a true hæmatoblastic crisis.

It is not easy to explain the fact that the increase in red cells may be slow during the first days of apyrexia, and that the number may even remain stationary or become lowered again for a few days. It is our opinion that, in some cases at least, it is due to persistence of the infection in spite of treatment, although in insufficient amount to cause fever. We know that in some cases of tertian cut short by quinine an increase in the elimination of nitrogen has been noted on the day of the expected attack, although the fever did not appear; probably for the same reason the number of red cells may become lessened, although there is cessation of the febrile attacks.

As is usually the case in secondary anæmias, the globular value tends to diminish during the reconstruction of the blood, because the red cells are reproduced more rapidly than the hæmoglobin; and it reaches normal only when the process is completed. One great difference between the process of repair in secondary and in malarial anæmias consists in the behavior of the leucocytes. We have already said that they diminish greatly during the course of malarial infection, while, as we know, there is leucocytosis in the anæmia from blood-letting, etc. Now, during the reparative process of secondary anæmias the leucocytes gradually diminish to normal, while in malaria they tend to increase to normal.

To these data should be added the observation of Dionisi, who found that the presence of crescent forms alone did not cause anæmia, nor did it disturb the processes of repair if these were under way. The same is true in the case of the pigmented forms of tertian fever, sterile in the human organism, and now regarded as gametes.

We have seen how the number of red and white cells is modified during an acute infection and during the period of reconstruction.

There remain to be seen the observations which we possess in regard to the variations in the hæmoglobin, the density of the blood, and the istonia of the red cells.

The *variations in the amount of hæmoglobin*, studied by Rossoni, who followed Vierordt's method, are, as a rule, the same as those in the number of the red blood cells. Under some conditions, however, and especially during the reconstitution of the blood, the globular value, that is to say, the amount of hæmoglobin in proportion to the number of red cells, may be less than normal, as usually occurs during the repair period in secondary anæmias. Rossoni says, in this connection, that the reconstruction of the hæmoglobin is apt to be slow, even after careful feeding, the administration of the specific remedy, and the use of iron. For instance, in a boy of eight years, twenty-five days after a pernicious fever, the amount of hæmoglobin was still only half of the normal.

As to the relation between the loss of hæmoglobin and the severity of the infection, Rossoni states that, as a rule, such a relation is evident, as we have seen in the case of the number of red corpuscles. Yet there are cases in which the gravity of the pernicious fever is not in proportion to the loss of hæmoglobin and of red cells. This is easily understood, if we take into consideration all the facts known in regard to the pathology of pernicious infections. We must also bear in mind that probably not all the red corpuscles in which we find young parasites are destroyed in the cases under active treatment. For instance, we once saw a youth suffering from soporous pernicious fever, in whose blood were found many non-pigmented estival parasites, and who recovered without diminution of hæmoglobin to the extent that we had been led to expect by an examination of the blood. Under the action of quinine many parasites leave or become detached from the corpuscle, which then preserves its vitality. We may add, however, that there are pernicious fevers in which anæmia is the symptom most threatening to the patient; this has been called by us anæmic pernicious fever.

The *variations in the density of the blood* are intimately related to those in the number of corpuscles; all authorities, in fact, agree that the specific gravity of the blood is in direct relation to its richness in corpuscles, and still more to the amount of hæmoglobin in the red cells. The researches of Viola, who worked according to Landois' method, have given results which are in perfect accord with what has been stated above in regard to the numerical variations of the red cells. Viola ascertained the degree of density in fifty cases of malarial fever. From his observations we learn in the first place that the malarial infection in itself exerts so profound a perturbation upon the

blood mass as to conceal the effects of such other disturbing causes as may act during the attack. This is true in regard not only to changes in the density, but also to variations in the number of the red cells. In spite of all the factors which tend to modify the relative proportion between the various components of the blood and therefore to affect its specific gravity (vomiting, diarrhœa, profuse sweating, and polyuria increasing it, and copious drinking, epistaxis, etc., diminishing it), and in spite of the hyperthermia (which in itself causes an increase in the density) the influence of the parasites upon the blood mass is so great as to make us believe that the constant variations in the density of the blood are chiefly in relation to the destructive action of the parasites on the one hand, and to the compensating action of the hæmatopoietic organs on the other.

Diminution in the density may begin at the onset of the febrile attack, even before the occurrence of chills; but it is more marked later. The maximum lowering at this stage was seen by Viola in a case of estival fever in which a specific gravity of 1.060 decreased after the chill to below 1.055. In the stages of fever and of sweating the decrease continues, but much more slowly. With the exception of pernicious fevers, the total decrease for a whole attack, due to a loss of red corpuscles, was found to be at the maximum 6.20 degrees. In one case of pernicious infection with a large number of parasites, during about six hours of fever the density fell 10.83 degrees.

Generally speaking, we may state that the density is modified in relation to the loss of red corpuscles. Viola observed that the greatest decrease is noticed in full-blooded persons, in primary affections, and during the first attack. Much less decrease is noticed at a later stage, when anæmia is more pronounced, and the least of all occurs (after a febrile attack, of course) in chronic malarial patients with a large spleen.

The changes in the specific gravity, which correspond perfectly to the numerical variations in the red cells (great decrease in the first, and less in the subsequent attacks), act in accordance with this law even when the parasites appear to be present in equal amount in the first and in the subsequent attacks, and when the fever is equally high in all. Viola attempts to explain this by the theory that the destruction of red blood cells depends only in a minor degree upon the direct action of the parasites and to a greater extent upon their toxic products. The toxic influence would seem to be exercised chiefly at the beginning of the infection, and scarcely at all later. This would explain why, with an apparently equal number of parasites, serious diminution in the corpuscles occurs in the first attacks and a slight decrease only in subsequent attacks.

After a cure has been obtained by means of quinine, the specific gravity of the blood rises sometimes at once, sometimes after a delay of a few days. After a while it stops, especially if the organism has been weakened by a long duration of the infectious process, but it proceeds with rapidity if the infection has been short and the anæmia acutely produced.

The *variations in the resistance* (isotonia) of the red corpuscles during and after the malarial attacks have been studied by Viola with the methods of Hamburger and Mosso. Viola found an increase in resistance coinciding with the decrease in specific gravity after a succession of attacks. He explains this by the theory that the least resistant red blood cells are the ones to be especially the victims of the invading parasites. In malarial patients who have suffered many relapses in consequence of which they are greatly debilitated, in whom there is neither great loss of red corpuscles during the attacks nor marked increase in specific gravity in the apyrexia, he found the resistance to be within normal limits. An increase occurs in the periods in which repair is active, which suggests the idea that the young red cells are the most resistant. Researches into this subject are still somewhat few in number.

Anæmia in Malarial Cachexia.—The degree of anæmia which may be found in chronic malarial patients and in cachectics is very variable. There are some patients with a large spleen, and fever recurring at long intervals, even of a month, and promptly checked by quinine, the number of whose red corpuscles is not below normal. This is especially the case in persons who live under good hygienic conditions, whose diet is good, and who are not subjected to excessive fatigue. The losses incurred during the occasional febrile attacks are compensated for during the long periods of apyrexia.

As a rule, however, patients who fall into a state of chronic infection after a series of insufficiently treated attacks, as in the case of farm laborers, give all the signs of the chronic anæmia of malaria. To the action of the malaria itself is superadded that of malnutrition and of overwork. In these cases the red corpuscles may be decreased to 2,000,000 and in cachectics to even 1,000,000 per cubic millimetre.

The circumstance quoted by Kelsch is worthy of note, that in these cases of chronic anæmia the number of red corpuscles is scarcely, if at all, lowered any further after the febrile attacks; sometimes it even rises a trifle, in spite of fever recurring at more or less long intervals. The red cells present quantitative oscillations due to the changes in reproduction and in destruction, yet the deficit is usually small, even when the attacks are grave by reason of high temperature and the general symptoms.

But, although the loss is small, the reconstructive process is apt to be slow, cachectics often remaining with a reduced number of red cells for several weeks, in spite of rest and regular treatment with quinine and iron. In our opinion, this is the result of insufficient work on the part of the bone marrow, by reason of which the quantitative balance, so to speak, of the red cells is maintained at a lower level than usual, the losses being compensated for up to a medium amount and no more.

The leucocytes are as a rule less than the normal. Out of thirty-three cases of cachexia studied by Kelsch, in twenty-three there were fewer white cells than normal, in nine the number ranged about normal limits, and in only one was there for a few day an increased number. In cachectics with a large spleen and with leucopenia, Kelsch has noticed that faradization of the spleen produces a decrease in the size of this organ, and a temporary increase in the leucocytes, even up to double or triple the usual number. One or two hours later, however, the spleen returns to its ordinary size, and the leucocytes again diminish in number, possibly falling even below the former figure.

This leucopenia of chronic malarial patients is connected with the deficiency in formative activity of the bone marrow, which also explains the extreme slowness of the return to normal in cases in which a perfect recovery occurs. We have seen above that the process of repair proceeds with some rapidity when the anæmia has been produced acutely, or when the infection has been of short duration; but evidently after several relapses the repetition of the damage to the hæmatopoietic organs affects their functional capacity. This explains why these chronic anæmias in some respects, in the leucopenia for example, more nearly resemble the so-called pernicious anæmias than they do secondary anæmias.

While in chronic malarial anæmia the special symptoms of the chronic malarial infection and of the cachexia predominate, and the anæmia is related to the persistence of the infection, there are cases in which, in spite of the cessation of the malarial fever, the anæmia not only persists but tends progressively to increase. These cases, which are somewhat rare, we have placed in the group of *post-malarial anæmias*. Bignami distinguishes in them various types according to the changes in the bone marrow, and the hæmatological conditions. (For the first see the section on Pathological Anatomy; for the second, that on Chronic Malaria.) In this connection we will merely say in regard to the pathogenesis of these post-malarial anæmias, that, although they develop after the malarial infection, we cannot regard them as due exclusively to the previous disease; indeed,

in these cases, the anæmia acquires a certain independence and goes on of itself when the factor which occasioned it in the first place has ceased to act. This suggests the thought that other factors, some of which are unknown, may take a part in the causation of the anæmia.

Among the conditions which aggravate the anæmia and contribute to its continuance after the infection has ceased are age, malnutrition, overwork, pregnancy, nursing, etc. In our hospitals it is not a rare thing to see a progressive and grave anæmia during pregnancy in women who were anæmic before they had malaria. Bignami has recently called attention to the fact that the fœtus is not so injuriously affected by these post-malarial anæmias as we might imagine *a priori*. In fatal cases, we are surprised to find the viscera of the fœtus relatively rich in blood and without alterations, especially malarial lesions, while the viscera of the mother are profoundly anæmic, and present the lesions characteristic of a malaria but recently extinguished. These facts might be adduced against the theory of the toxic origin of these malarial anæmias held by some authorities, unless we wish to believe that the hypothetical anæmia-producing poison is impotent to disturb the hæmatopoiesis of the fœtus, or to injure its red blood cells. But in our opinion, a study of post-malarial anæmias leads to the belief that they are above all due to the alterations which pathological anatomy reveals in the bone marrow, namely, megaloblastic degeneration, and atrophy of the hæmatoblastic marrow. The condition of the medullary function almost exclusively governs the course of these anæmias.

A microscopical examination of the blood of malarial anæmic patients shows changes varying with the gravity of the anæmic condition, and according to whether it follows an attack at once or after some time, or during the period of repair, etc. We do not here refer to the changes due to the invasion of parasites (presence of parasites, pigment, etc.), but only to those due to the anæmic condition which is the result thereof.

Marchiafava and Celli from their earliest researches (1883) called attention to the presence in malarial patients of nucleated red cells, which they found in acute infections (sometimes) and in convalescence during the period of repair, of macrocytes, and of the red cells which are colored in an abnormal manner by methylene blue. They considered the last named to be young red cells which had only recently lost their nuclei.

The nucleated red cells usually belong to the normoblasts, but only exceptionally in some cases of anæmia which were on the way to recovery, we have seen a few megaloblasts together with the nor-

moblasts. In rare cases forms of normoblasts in karyokinesis have been seen in the circulating blood. The nucleated red corpuscles are usually numerous in the anæmic conditions which are acutely produced after the infection has lasted a short time only; or in those cases in which the reparative process proceeds rapidly and sometimes by a true hæmatoblastic crisis. On the other hand they are found in small number or not at all in cachectics.

The macrocytes (pale red cells, two or more times the usual size) are found in large numbers especially in cases of profound anæmia, as Kelsch observed as early as 1875. Poikilocytes may also be present, but rarely possess the importance that they do in other kinds of anæmia.

As to the leucocytes, in addition to the decrease in their absolute proportion, to which we have referred, there is usually a relative increase in the uninuclear cells, especially the large ones. In only two cases of pernicious fever have we seen a few medullary eosinophile cells, such as are found in leukæmia; this is altogether exceptional.

In grave post-malarial anæmia, a microscopical examination of the blood gives varying results. In some cases there are no nucleated red cells at all, and these are in all probability the cases in which at the autopsy the marrow of the long bones is found to be yellow, and that of the spongy bones poor in hæmatoblasts; in other words there is atrophy of the marrow, even of those bones the function of which is usually dormant in the adult. In other cases there are scanty normoblasts, as in the ordinary anæmias of chronic malaria. Finally in some, an examination of the blood shows alterations like those of pernicious anæmia, and the presence of megaloblasts. In all these post-malarial anæmias there is, as a rule, a leucopenia with relative increase of the uninuclear leucocytes, the intermediate forms, and the lymphocytes, and a notable diminution of the ordinary leucocytes with polymorphous nuclei and neutrophile granulations.

From the many data so far given (numerical variations of the red and the white cells, quantitative alterations in hæmoglobin, modifications of the resistant powers of the red cells, alterations visible on microscopical examination, etc.) the general character of the acute and chronic anæmia of malarial patients can be clearly appreciated.

Every attack of malarial fever is followed by a diminution in the number of red and white corpuscles, and in the amount of hæmoglobin, a decrease which may be very marked; at the same time the specific gravity of the blood diminishes and the resistance of the red corpuscles tends to increase, the explanation of this probably being that the least resistant corpuscles are destroyed.

This decrease in the corpuscular elements, which may be marked

in the first febrile attacks, tends to become constantly lighter, and is very slight after febrile attacks in chronic malarial patients. The reconstruction of the blood during apyrexia, which proceeds actively at the beginning of the infection, although less so than in other secondary anæmias, becomes progressively slower, and is slowest of all in chronic malarial patients after a series of relapses. Thus is established a condition of chronic anæmia which requires a long time for its cure.

In the cases in which after cessation of the infection reconstitution of the blood does not occur and the anæmia becomes progressive, there is probably exhaustion of the medullary function on the one hand, and continual action of other causative factors, as pregnancy, malnutrition, etc., on the other.

It is worth our while to dwell upon a fact to which we have already several times referred, and that is, that under apparently similar circumstances a malarial patient recently infected loses more red corpuscles after an attack of fever than does a person already anæmic from repeated relapses. This cannot be due to rapid compensation, because we know that in relapses the process of repair occurs more slowly than in the primary attacks, and even in these more slowly than after hemorrhage. Everything leads us to think that in the relapses a relative immunity is established by means of which there is a lessened decrease of red corpuscles, although the fever may be high and the symptoms severe.

Concerning the mechanism of this immunity we can say but little. Is it a relative immunity of the red cells owing to the direct action of the parasites upon them? Some have said that even when the number of parasites was apparently equal in a primary case and in a case of relapse, the loss of red cells was greater in the former than in the latter. If this were true, we should be forced to the conclusion that in the relapse not all the parasite-infected corpuscles are destroyed; and we might be led to think that some of them recover, so to speak, from their infection, in consequence of the exit of the young parasites. We have ourselves noted this exit with preservation of the red cell as a result of the action of quinine. We have, for instance, seen a young man who was already anæmic, attacked by pernicious fever, with innumerable young parasites in the blood, and yet who recovered from the infection without having a decrease of red corpuscles to anything like the amount that we should have expected from the enormous number of parasites found at first. We have further noticed that in the blood of patients under large doses of quinine, we may find free in the blood many young parasites which are perfectly recognizable in preparations stained by Romanowsky's method.

The explanation proposed above would be acceptable enough were it not that it is difficult to place implicit trust in the figures which we are able to give in regard to the number of parasites found in the blood, the sources of error in the matter being so many.

May one acquire immunity against the hypothetical anæmia-producing toxic action of the parasites? We have already, in previous writings, expressed our doubt of the production of a corpuscle-destroying toxin by the development of the malarial parasite, having noted that we do not find in malaria that alteration of the red cells, especially the so-called hæmoglobinæmic degeneration, which is the constant result of all or nearly all blood poisons.

Nothing, however, forbids a belief that a special toxin of parasitic origin causes destruction of the red corpuscles by a specific action of its own. On the other hand, we have endeavored to explain the particular form of early necrosis which occurs in the red cells in estivoautumnal fevers (brassy bodies) by supposing that they result from a toxic action exerted upon them by the parasites. For this reason, we cannot altogether repudiate the theory advanced above, although the researches so far pursued with the purpose of directly proving the existence of poisons caused by the parasites have given negative results. We would indeed note that, given the theory of a globulicidal toxin, the relative immunity in relapses and in chronic malaria would be perfectly explained. We know, in fact, that up to a certain point this immunity can be acquired by animals treated with hæmolytic poisons; for instance, in dogs poisoned by pyrodin, Bignami and Dionisi have seen that in the beginning of the experiment even after the administration of relatively small doses of the poison (20 to 30 cgm.) the red cells were in the course of several days reduced to about half their number. If the experiment was dropped, and then taken up again in the same animal after the number of red cells had almost or quite reached normal again, the small doses no longer possessed the corpuscle-reducing power that they had in the first experiment. The red cells had acquired a certain degree of immunity against the action of the poison.

In the same way, as it seems fair to infer, the patients in malarial relapses acquire a certain relative immunity in regard to the anæmia-producing action of malaria, whatever may be the theory as to the mechanism of this action.

We have now seen that a study of the biology of the parasites satisfactorily explains the decrease in the red corpuscles. But there is another property special to this form of anæmia, to which we would draw the reader's attention, and that is the marked diminution in the leucocytes, the reason for which we must endeavor to ascertain.

This leucopenia of malarial patients is observed not only in the ordinary acute infections, but also to a very high degree in chronic malarial and cachectic patients.

In the first place, in considering the action of the leucocytes in an acute infection, we are struck by the fact that they diminish as a result of the ordinary febrile attacks—in the quartan and tertian, as in the estivoautumnal—while for the most part they increase in number in pernicious fevers. We find, moreover, that in the ordinary febrile attacks there is sometimes a slight and transitory increase during and for a short while after the chill (Kelsch, Vincent). In seeking to explain the process, we must of course take this contradictory behavior into account.

It appears to us that the reason for all this is found in the physiological offices which are performed by the leucocytes in malarial infection. We saw in the preceding chapter that the leucocytes act with energy as phagocytes, taking up pigment, the residua of segmentation, free parasites, altered red corpuscles, etc., and we have stated it to be our opinion that these substances exercise a positive chemotactic action upon the white cells, especially upon the large uninuclear leucocytes. This granted, it is natural to infer that the white cells gather in those vascular areas in which are found the substances to be taken up, which, as we know, are set free in the blood chiefly in the act of multiplication of the parasites. We can therefore understand how, in a regular tertian, in which fission of the parasites occurs in every part of the vascular system, there may be a transitory increase in the leucocytes at the beginning of the attack —that is to say, during the hours in which fission is accomplished. With great rapidity, however, all the products taken up by the phagocytes are deposited in the internal viscera, spleen, liver, etc., whence the speedy diminution in the number of white cells.

In estival fevers, in which multiplication takes place almost exclusively in the internal viscera, we can understand that in these organs there should be an accumulation of leucocytes, because there are to be found those products which exercise a chemotactic action upon the leucocytes. During the course of a pernicious fever, on the other hand, we nearly always find adult parasites and even fission forms circulating in the blood, and at the autopsy they are often found accumulated, in the brain for instance, in enormous numbers; correspondingly, we find an increase of leucocytes in blood taken from the finger, that in the cerebral vessels, etc. In other words the leucocytes are found in those portions of the vascular system in which are found the substances which attract them. When at the end of the pernicious attack all the impurities in the blood, the pigment, necrotic

parasites, etc., are gathered in the spleen, liver, etc., as occurs to foreign bodies injected into the circulation, the leucocytes gather there too. Thus we can understand the leucopenia which, after the cessation of pernicious conditions, follows the increase sometimes found during the same. We can also understand the contradictory behavior of the leucocytes in pernicious and in ordinary estival fevers, in which there is leucopenia even during the febrile attack.

In the same cases of pernicious fever the increase in the number of leucocytes found in the peripheral blood is assisted by circulatory weakness, which may even be excessive; sometimes there is an apparent hyperleucocytosis in collapse. Thus the increase in leucocytes at the beginning of a grave attack, in the cold stage, may be only apparent and related to the slowing of the circulation, which is manifested by cyanosis of the lips and the extremities. In one case under special individual conditions, we witnessed the following occurrence: the patient was a youth suffering from mitral insufficiency, who, while crossing a passage in the hospital of Santo Spirito, was affected by sudden weakness and fell to the ground. He was put to bed, where it was found that his pulse was very small, and his extremities were cold and cyanotic; consciousness was perfect; the rectal temperature was 38° C. (100.4° F.). As he came from a region of grave malaria, an examination of the blood was made, and there were found many young parasites, many in process of multiplication, and many pigmented white corpuscles; but the most striking fact was the enormous number of leucocytes, a number so great as to cause at first thought a suspicion of leukæmia. About half an hour later, the patient having been restored by injections of caffeine and ether, the proportion of white corpuscles had returned to normal; later, at the end of a febrile attack, there was the usual diminution of leucocytes characteristic of malaria. In this case, the special condition of the circulation in the patient exaggerated the altered distribution of leucocytes which are usually found during the course of an attack.

In our opinion, therefore, leukopenia during an acute infection does not depend upon a real diminution in the number of leucocytes, but upon a change in their distribution caused by the tendency of the white cells to accumulate in the vascular areas in which the blocks of pigment, mature parasites, etc., in short, the substances which they are to take up, accumulate. In the second place, the great destruction of leucocytes which follows their phagocytic action contributes greatly to maintain this leucopenia of the circulating blood after the attacks have ceased. An anatomical examination shows a large number of degenerating or necrotic pigmented leucocytes in the spleen and in the liver.

The leucopenia found in anæmic persons after repeated relapses, and in cachectics, can be explained only by taking into account another factor—the diminished activity of the hæmatopoietic organs, especially the bone marrow. This deficient activity in the marrow is well shown in those cases of post-malarial anæmia in which we find the marrow to be yellow or gelatinous, or serous, in spite of the grave condition of anæmia. But even in the ordinary cases of anæmia from malaria, we can deduce it from the modications in the numerical proportions of the various kinds of leucocytes. We find in these cases a relative increase of large uninuclear leucocytes and of lymphocytes, and a diminution of the cells with polymorphous nuclei and neutrophile granulations, just as we do in anæmic conditions with diminished activity of the medullary function. The increase in the proportion of lymphocytes, which is sometimes very great, might also be thought to be in relation to the chronic enlargement of the spleen. It is well known that the suppression or diminution of the splenic function determines a lymphocytosis, as Kurlow was the first to observe in animals; and pathological anatomy shows that in chronic enlargement of the spleen there are such alterations of the splenic parenchyma as to give a good foundation for the belief in the diminished physiological activity of this organ.

From what has so far been shown, it is evident that malaria alone never produces a leucocytosis in the proper meaning of the term, or a transitory increase in the number of neutrophile leucocytes with polymorphous nuclei. We find a leucocytosis only under special conditions, as in a state of collapse, or at the time of death, or after profuse diarrhœa, or from complications. Infection by the pus microbes, or by the pneumonia diplococcus is the most frequent cause of this leucocytosis.

The Febrile Attack.

There were numberless theories among the older physicians as to the genesis of intermittent fever, founded upon the then prevalent ideas of physiology and pathology. It would be a futile task to recount them here, and we need only mention that among them were some which indicate the limit to which such hypotheses based upon known facts could go. Thus some endeavored to explain the intermittence of the fever by the supposition that the pyretogenous ferment entered at regular intervals into the blood, from which, after undergoing modifications, it was eliminated, whence the apyrexia. If this elimination was delayed, or if the pyretogenous substances accumulated in the blood, the subintrant and subcontinuous forms were

caused (Torti, Borsieri). Sydenham thought that accumulation in the blood of the materia peccans was the true cause of the febrile attack, the hot stage signifying a reaction of nature against this substance in order to facilitate its expulsion, which occurred during the sweating period. The return of the paroxysm occurred because some of the substance which was undestroyed, after remaining latent for a period varying with the type of the fever, returned in increased amount to provoke a new reaction in the organism. As to the cause of the diversity in febrile types, in which the development of the materia peccans up to the point of provoking an attack takes sometimes one and sometimes two or three days, Sydenham pleads ignorance, as he does in regard to the succession of other natural facts. He adds: "Quippe cum persuasissimus sim naturam non minus hic loci quam in alio quibuscunque, methodo quadam certa atque ordine progredi; quartanosa enim (ut sic dicam) ac tertianosa materia non minus naturæ legibus subjacet, iisque regitur quam alia corpora qualiacunque."

This notion of the older physicians as to the reason of the fever and of the intermittence was modified by later writers. For the alteration of the blood as a cause of the intermittent fever was substituted irritation of the cerebrospinal centres; intermittent fever was thus a cerebrospinal neurosis. Maillot (quoted by Laveran), who earned well-deserved praise for his treatment of malaria in Algeria, defined the disease as a cerebrospinal irritation, "the active and hyperæmic irritation of the cerebrospinal axis is that which constitutes the nature and essence of the intermittent fevers whether simple or pernicious." Laveran, indorsing this view, adds that the parasites directly irritate the nervous centres, whence the chill and the other symptoms of both simple and pernicious intermittent fevers.

As to the cause of the intermittence, Laveran premises that intermittence is neither a constant nor a specific characteristic of malarial infections. He does not acknowledge the importance of Golgi's law in regard to the parasitic cycle, and he holds that the intermittence is due in part to the phenomena of phagocytosis, in part to the varying activity of the parasites in different climates, and in part to the variability in the reactions of the human organism.

Two tendencies therefore existed in medical science for a considerable time. The first, and oldest, was to connect the periodical fever with the special properties of the producing cause of the attack, that is to say, with the property of the *materia quartanosa* or *tertianosa* as Sydenham expressed it. The second, of more recent date, represented in our day by Laveran, was to refer the salient characteristic of malarial fever, which is its periodical intermittence, to an

altered functioning power of the diseased organism, particularly of the nervous system. The clearest expression of the first mode of thinking is found in the words of Rasori which we have quoted elsewhere: "Several years ago I gave it as my opinion that the intermittent fevers are produced by parasites, and that they renew the attacks in the act of their reproduction, which follows with more or less rapidity according to the various species" (1846).

The recent discoveries in regard to the malarial parasites and their life cycle have shown that the theories of some of the older writers (Sydenham) were not erroneous, and that the words of Rasori represent a happy intuition of the truth; for the fever is a result of the parasitic blood dyscrasia, and the various occurrences during the fever are related to the development of the parasite.

The recent researches of Golgi have demonstrated (1) the relation between the cyclical development of the parasites and the periodical succession of febrile attacks, so that an attack corresponds to the multiplication of a group of parasites; (2) the constant relation of the individual attacks to the development, maturation, and reproduction of a generation of parasites. Thus, the quartan is produced by a generation of parasites which develop in the space of three days, and the fever coincides with their multiplication; whence the succession of attacks separated by two days of complete apyrexia. The tertian is determined by a generation of parasites which develop in two days, giving attacks separated by one day of apyrexia. Later it was recognized that the difference in the length of the cycles of development of the quartan and of the tertian is one of the stable characteristics of species, the parasites of the quartan and tertian being different stable species of malarial parasites.

These facts, which can be studied with the greatest ease in blood taken by puncture from the finger in a quartan or tertian infection, were soon confirmed by the great majority of clinicians and pathologists.

Next Marchiafava and Celli demonstrated that the same law holds good for the estival fevers in general; and Marchiafava and Bignami, as a result of special researches into the summer-autumn tertian, found that in this type also the attacks occur at the moment when the parasites are multiplying. Only in this group of fevers the fact is less easy to ascertain, because, as we have seen, the life cycle of the parasites is partly completed in the internal organs. If, however, we puncture the spleen in the first hours of the attack, we usually find in the blood parasites with central pigment, in which the nuclear division has already taken place, and forms of completed sporulation. Thus for all the kinds of malarial fever the law holds good

that the parasites determine the attack coincidently with the act of their multiplication.

Various theories have been held regarding the mechanism by which the fever is produced. Golgi, after his earliest researches, first supposed that the cause of the fever was to be found in the entrance of young parasites within the red cells, an event which follows quickly upon multiplication. But Antolisei opposed this theory; he observed that in quartan fever, if quinine is given a few hours before the expected attack, the latter is not prevented, nor is the sporulation of the adult parasitic generation interfered with, but during the attack no young parasites are seen within red corpuscles; therefore he concluded that the attack is produced as a result of the dispersion of the spores in the plasma, by a mechanism intimately connected with the process of multiplication, even if the young parasites do not invade new red cells.

But when sporulation is accomplished, and the spores are dispersed in the plasma, it is reasonable to suppose that chemical products endowed with pyretogenous properties are also poured into the blood. Golgi and Baccelli upheld this view, which has been adopted by the majority of the authorities (Thayer, Mannaberg, and others). In its support we ourselves adduced the analogous fact that various alterations produced during the course of the infection, especially in the grave fevers, are to be regarded as of toxic origin; such, for example, are brassy degeneration of the red cells, some degenerations and necroses of the kidneys, etc. Other writers have added that the increased toxicity of the urine in malarial patients is an argument in favor of the toxic origin of the febrile attacks.

But, although many things seem to bear out the hypothesis, we must acknowledge that so far there is no solid basis of facts for it to rest upon. So far as we know, no one has succeeded (we have in mind several experiments of Celli) in demonstrating that the serum of the blood of a malarial patient, withdrawn during the attack, has the special property of raising the temperature of a healthy person to any extent. Even if this were so it is questionable whether we could, without further proof, assume that this hypothetical pyretogenous toxin is a product of the biological activity of the parasite. We might, for example, suppose that it came from the red corpuscles, the residua of which are scattered in the blood as soon as the parasites multiply; or finally, we might assume that the pyretogenous toxin comes from both parasites and disintegrated red cells.

The knowledge at our command does not permit of our carrying our analytical studies up to this point. We may remark, however,

that even in the case of the pathogenic microorganisms of other diseases, it has not as yet been found possible to determine accurately the nature and the origin of the substances producing the fever. Even in the case of microorganisms which can be grown in some ordinary culture medium, it is not absolutely known whether the pyretogenous substance or substances are manufactured directly by them or come from the culture medium in which they have grown and which has been modified by the process of their development. Indeed, even in those cases in which research is facilitated by artificial cultures we are not able with any certainty to separate the bacterial secretions from the products derived from the culture media, nor these from the products of the many bacteria which die and which are disintegrated in those media. From this point of view, the problem of the genesis of the malarial febrile attack is therefore no further advanced than that of the fevers produced by pathogenic schizomycetes. Therefore, to assume that the malarial parasites during their multiplication pour out a pyretogenous secretion into the blood is to state a theory which is probable, but which, owing to the absence of convincing data, it is at present profitless to discuss.

In malarial fevers, however, we possess the great advantage of being able to ascertain, by a systematic examination of the blood, with what moment in the life cycle of the parasite the febrile attack coincides. We cannot describe the mechanism by which the fever is produced, but we can state *what are the conditions under which the pyrexia begins and is developed.*

From the possibility of this analytical research we see the importance which the study of malarial fever possesses even as regards the general pathology of infective fevers.

The three classical stages of the febrile attack typical of malaria are well known. Now modern research permits us to demonstrate schematically what occurs in the blood during the cold, the hot, and the sweating stages, that is, to the end of the attack. At the onset we find in the blood parasites in process of multiplication or which have already multiplied, and the young parasites, making their exit from the red cells, become free in the blood. At the same time the residua of the red cells disintegrate in the plasma, the specific gravity of the blood is lowered, the pigment of the sporulating bodies becomes free in the plasma, and with it the so-called residua of segmentation; these both are taken up by phagocytes, and with them some parasites in process of multiplication. Some adult parasites degenerate in place of going on to multiplication. In the quartan and tertian nearly all these occurrences take place throughout the vascular system, so that it is easy to watch each part of the process; in the summer-autumn

fevers, on the other hand, all this is accomplished chiefly, if not exclusively, in the vessels of the internal organs.

As is well known, in the quartan and tertian fevers the pyrexia is ushered in by a more or less prolonged chill—long continued and exceedingly distressing in the quartan—which is produced when the central temperature has already begun to rise (De Haen). Even before the occurrence of the chill there is a contraction of the cutaneous vessels which attains its maximum in the period of the highest elevation of temperature, and then diminishes. In the estival fevers, on the other hand, the chill is often absent. This cannot be due to the high temperature of the atmosphere, as some have claimed, because even in the heat of summer the chill in a quartan attack is very severe; nor as others have maintained, to the rapidity with which the temperature rises, for in some cases of estival tertian without initial chill, the temperature may rise with great rapidity. From the parasitic standpoint there is a fundamental difference between the tertian and quartan and the estival fevers, and that is that all these changes which occur in the blood at the beginning of the attack occur in the tertian and quartan throughout the vascular system, and can be seen even in the vessels of the skin, whereas in the estival tertian they occur in the vessels of the internal organs under relatively stable conditions, and the parasitic forms which finally produce the fever are not circulating. In our opinion this fundamental difference may be regarded as bearing upon the occurrence or non-occurrence of the chill. The latter may be looked upon as the final effect of a local reaction of the cutaneous vessels, which contract with energy when the substances derived from the multiplication of the parasites are set free in them, and which do not contract to the same extent when the whole process occurs in certain viscera, as the spleen, etc.

As the attack advances we continue to see in the circulating blood the young parasites which at first adhere to and then penetrate into the red corpuscles; at the same time all the products taken up by the phagocytes are gradually being deposited in the internal organs.

Towards the end of the attack the number of young endoglobular parasites attains its maximum; we must therefore suppose that all which have not been taken up by the phagocytes have become guests of the red cells. At this point the fever ceases, and the endoglobular parasites continue to develop within the red cells during the period of apyrexia, until, having reached maturity, they multiply and cause a new attack.

The schema, which can be applied to every kind of malarial fever, may be summed up as follows: The fever begins during the period in which the parasites multiply and issue as young forms from the

red corpuscles, and ceases when all the young parasites having in their turn been taken up by red corpuscles begin within them their life cycle.

The time required for the development of a young form up to multiplication (sporulation) corresponds to the interval between the attacks, and because the duration of this period differs according to the different kinds of malarial parasites, and is within certain limits constant for certain species, we have the several fundamental types of malarial fever: quotidian, tertian, and quartan.

But another indispensable condition of the occurrence of an intermittent fever, as a typical quartan or tertian, is that all the parasites shall be found at about the same stage of development, so that all or nearly all shall reach maturity and sporulate at about the same time, or in a relatively short time; in other words it is essential that there should be only one generation (as the usual expression has it) or one group of parasites in the blood. This is in fact what occurs in the regular intermittent fevers; and it is really remarkable to see, for instance in a typical quartan, how nearly all the parasites are sporulating during the stage of the chill. This tendency of the parasites to form in line, as it were, finds its counterpart in what is known of the processes of multiplication in other organisms which, under determined conditions, multiply contemporaneously. For example, there are the "conjugation epidemics" of certain protozoa; the infusoria kept in one culture are not preserved for a long time by multiplication by division, but the single individuals end by showing changes in their nuclear apparatus, cease to divide, and finally become exhausted. To maintain the species, it appears to be necessary that at certain intervals two should unite. Now this usually happens at about the same time in all the organisms forming a culture, wherefore we speak of "epidemics of conjugation." It is of interest to note that these "epidemics" may be caused in a culture by diminution of the nutrient substance, and interfered with or sometimes even prevented by abundant nutrition, so that the single individuals die and the culture is extinguished. Although in the case of malarial parasites the process of multiplication is quite a different one, it is impossible not to see a certain distant analogy between it and the circumstances described above.

Even in some infections which begin with irregular fever we may observe that, after a certain number of attacks, the fever tends to become regular, and at the same time the parasites tend to multiply in distinct groups. A certain initial irregularity has frequently been seen in experimental infections (caused in man by the injection of malarial blood), especially in the estivoautumnal fevers, and even in

spontaneous infections the same thing not rarely occurs. But after a while the parasites manifest the tendency to form in line.

The condition alluded to above (progressive development of the parasites, and simultaneous multiplication) is not a constant occurrence. In some fevers, especially in the estivoautumal, even when there is only one generation of parasites present in the blood, the parasites are ordinarily not found in the same stage of development with the regularity that they are in the quartan, for instance, and thus the multiplication of the single individuals does not occur in a short space of time, but successively and in groups, and continues for a variable time, which may be twenty-four hours. This biological peculiarity, judging from our researches, accounts for the prolonged attacks which are the rule in estival tertian fever.

It may moreover happen that during the whole course of the disease parasites in various stages of development will be found in the blood at the same time—young parasites, growing parasites, and those in sporulation. When this is the case, we can understand that we shall not have attacks which are distinctly intermittent, but *continuous and irregular fevers*. And this in fact occurs with all the species of parasites, but especially in the fevers caused by the estival parasites.

We have said that the duration of time necessary for the completion of the entire life cycle of the parasites is, within certain limits, constant for each variety. And because in this fact resides the origin of the febrile type, it follows that *the febrile type is within certain limits constant for each species*. Thus, while it is not rare to see an anticipation or a retardation of several hours (anticipated or retarded attacks) we never see a quartan become a tertian or vice versa; or, at least, when this does occur, an examination of the blood will show that the quartan parasites which have disappeared have been replaced by the tertian, or vice versa. We have to do, therefore, with two infections, one succeeding the other. The same thing may be seen when an estival fever is followed by an ordinary tertian, etc.

The opinion formerly held by some medical writers that the febrile type may vary was based upon the fact that we often see a quotidian change to a tertian, or a quotidian followed by a double quartan or quartan, or vice versa. But after Golgi had described the quartan and tertian parasites it was easy to see by an examination of the blood that many fevers which have the clinical type of a quotidian are only double tertians, and others triple quartans; that is to say, we have two kinds of quotidan, one of tertian origin and the other of quartan origin, to which we must add a third species, the estival quotidian. In the quotidians of tertian origin we find two genera-

tions of tertian parasites which come to maturity with about one day's interval; in those of quartan origin three generations of quartan parasites, which mature successively with about a day's interval between them. A knowledge of these facts explains the apparent variations in the febrile types seen in practice; thus a quotidian of tertian origin may be followed by a tertian, when one generation of parasites ceases to develop and the other continues. In the same way a quotidian of quartan origin may become a double or simple quartan, when of three generations of parasites only two or one continue to develop. It is also known that within these limits the variations in type may be produced artificially by means of quinine, which, given a few hours before the beginning of an attack, may suppress only one generation of parasites, leaving those of the other generation which produce the next attack.

It is a fact of the greatest interest that we often find in a patient two generations or groups of tertian parasites, or three generations or groups of quartan parasites, which mature regularly with about a day's interval between them, giving in either case a quotidian fever. We know that these quotidians of tertian or quartan origin are often primary infections, in which the type later becomes simplified, turning into a pure tertian or quartan. But it is also surprising in some cases to see the quotidian of tertian or quartan origin relapse in the same form after a variable period of apyrexia; the several generations of parasites awaken, so to speak, after a period of latency, and develop with their previous regularity. In other cases the primary infection may be a simple tertian, for example, and only in a relapse becomes a double tertian; but the variety given above is by far the more frequent, that is to say, a tertian which is double from the beginning. The same may be said in regard to the relations of the triple and double quartan with the simple quartan. We do not know that any one has given a satisfactory explanation of these facts.

Thayer endeavors to ascertain the mechanism by which a simple tertian becomes double, or, in other words, in what way a single group of parasites gives way to multiple groups. He observes that in a tertian at the beginning of a febrile attack we see the multiplication not only of the parasites which have attained complete development, but also smaller forms apparently of a less advanced stage, as if there were some influence which caused the multiplication of forms, which were not quite mature, at the time of sporulation of the chief group. But if the difference in development reaches a certain limit, if, for example, at the moment in which most of the parasites multiply there are some which are only half developed, these escape the supposed unknown influence, and become the nuclei for a parasitic

group which mature on the following day. But why should the second group of parasites which has its origin in this manner mature at about twenty-four hours' interval from the other? It is known, in fact, that the paroxysms in the ordinary tertian have a tendency to begin in the morning hours (between 8 A.M. and 1 P.M.). Now when the tertian becomes double, the new attack usually occurs at about the same hour that the first was produced. How can we explain this circumstance even admitting that the tertian is doubled by a retardation or anticipation in the development of some parasites? Nor do we obtain a satisfactory explanation from the hypothesis of Pes (*Riforma Medica*, 1893), who supposes that some of the organisms which were very small when they entered into the red cells mature more rapidly, and thus by early segmentation eventually form a second group.

These various interpretations of the phenomena do not, in our opinion, explain the regularity with which they occur—why a simple tertian, for instance, becomes quotidian with attacks occurring every day at about the same hour. This makes one think that the fact cannot be due to an anticipation or retardation in the development of certain parasites. Why should these anticipations or retardations be usually of just twenty-four hours and not more or less? And if we admit the possibility of an anticipation or delay in the development of the parasites of about twenty-four hours, how can we reconcile such a supposition with the regularity with which the attacks of a simple tertian are usually developed? It would, moreover, be absolutely impossible to explain, by the theory of an anticipation or retardation in development, the fact that a simple quartan may become triple, or why one generation of parasites is followed by three and usually not more.

It appears to us very probable that the cases in which a simple tertian is followed by a double tertian are those in which two groups of parasites have existed in the blood from the beginning of the infection, but one of them in insufficient quantity and of too feeble strength to excite a fever. This belief is chiefly based upon the fact that the cases of tertian, which are double from the beginning, are more frequent than those in which the double tertian is secondary to an apparently simple tertian; secondly, upon the fact that in the majority of simple tertians, in which the temperature is taken regularly in the days intervening between the attacks, we find a slight elevation which represents a rudimentary attack, so that nearly all simple tertians are in reality double tertians in effect. The same may be said in regard to the quartan and the triple quartan, which, as a rule, are primary infections.

The fundamental questions then, we believe, are why there are very often cases of primary infection produced by various groups of parasites; why these groups usually mature regularly with about twenty-four hours' interval; and finally why there are usually not more than two of them in the tertian (double tertian) and not more than three in the quartan (triple quartan), the number being greater only in altogether exceptional cases. *A priori* we should expect that infections with many generations of parasites maturing at irregular intervals would be, if not the rule, at least of frequent occurrence; but as we have said, this is not the case. Why do patients often have one, two, or three groups or generations of quartan parasites sporulating at about one day's interval, or one or two generations of tertian parasites, and scarcely ever a larger number? In other words, why are the irregular fevers of quartan or tertian origin exceptional as compared with the single, double, or triple quartan, and the single or double tertian?

At first thought it is hard to understand why a person living in a malarious region is not continually infected by malarial parasites. It seems to us probable that the matter under discussion depends upon the mechanism by which the infection is taken. As we have seen, the malarial parasites are inoculated by special mosquitos which bite usually in the evening twilight and at night. Now, let us suppose that an individual sojourning in a malarious region is bitten every night by infected mosquitos, and consequently inoculated with quartan parasites; after the first three nights he will have in his blood three generations of quartan parasites, which, having been introduced at intervals of about a day, will mature at the same intervals; the result will be a triple quartan. If then, the same individual continues to be bitten and inoculated with quartans in the following nights, it is evident that the new generations will be superimposed upon the first, and join in with them, and the fever will consequently remain a triple quartan, without irregularity or complications. The same deductions may be made in case of the other fevers of regular type. In conclusion we may say that the presence in the patient of several groups of parasites maturing at more or less regular intervals of twenty-four hours can probably be explained by the fact that the inoculation of the patient by malarial mosquitos usually occurs at the same interval of time.

A double tertian may later become single, or a quartan which is triple in the beginning may change into double or single, either spontaneously or as the result of treatment. We have already said that quinine given at the right time, in rather small doses, may suppress one group of parasites, leaving the others more or less undisturbed.

When this occurs spontaneously, we cannot but think that there may be attenuation of one or two groups of parasites, which temporarily lose the power of causing a febrile attack. It is extremely instructive to follow a triple quartan which becomes single; in such a case, we have seen two generations of parasites become weakened in the blood and produce no more fever, while one continued to develop and to give regularly the attacks of a simple quartan. In spite of this, during the stage of apyrexia we have continued to see for several days even a few quartan parasites in the various stages of development belonging to the generations whose activity of growth had been weakened. The phenomenon must therefore be considered as an attenuation related to the immunity which the patient slowly acquires; it is in fact observed only in patients who have suffered a long while from the infection, and who begin to show a tendency to spontaneous recovery. Regarding the several febrile types in respect of which there have been no recent investigations, such as the quintan, sextan, etc., and the long-interval fevers, we refer the reader to the section on the Classification of Malarial Fevers.

Malaria without Fever.—In some patients all the conditions described above may exist and become manifest when the disease appears, and yet there may be no elevation of temperature. This occurs chiefly in two classes of cases: (1) in some extremely grave infections with pernicious symptoms, and (2) in certain patients in whom the disease tends to a spontaneous cure.

1. The first class has long been known. All physicians are aware that there are grave and sometimes mortal infections in which the rectal temperature may be little above the normal or may even be subnormal. When the blood is examined in some of these cases, we may find many parasites in fission, numerous phagocytes, etc., so that the conditions which produce pyrexia are there without its being produced. The reason for this can be found only in special conditions of the patient's organism; just as there may be a lobar pneumonia without elevation of temperature, especially in aged and feeble persons, so there may occur grave malarial infections without fever.

We recall the case of a man of somewhat advanced years who was brought to the hospital of Santo Spirito in an algid state; in the blood there were numerous parasites in all the stages of development. He was cured by the use of quinine and stimulating remedies, but after about three weeks the disease returned, and during this relapse there was the same algid state with a subnormal rectal temperature; the same thing was also observed in a second relapse. In this patient every malarial attack was accompanied by profuse diarrhœa.

Evidently the reason for these phenomena lay in some individual conditions not thoroughly known.

2. A similar phenomenon occurs, as we have said, when, usually after long duration of the infection, the patients have acquired a certain degree of immunity. This may be well studied in the quartan, in which, as we know, the whole life cycle of the parasite takes place in the circulating blood. In some patients we can see that even after spontaneous cessation of the attacks the parasites go on to the completion of their life cycle, multiplying with regularity, but even though fission and a fresh invasion of parasites occur, the febrile attack is absent. We might say that the fever is absent because the parasites, and especially the multiplying forms, are too few in number to cause fever, but this supposition is met by the fact that there are primary infections in which, with an even smaller number of parasites, severe febrile attacks occur. We might also suppose that the parasites were so modified as to lose their capacity for producing the pyretogenous material in sufficient amount to produce the fever. But even this is rendered untenable by the fact that if the person under the above-mentioned conditions, with parasites circulating in the blood, is exposed to one of the occasional causes of malarial infection, as for instance cold, he may have the febrile attack. We have seen patients in whose blood quartan parasites regularly completed their life cycle without occasioning fever, but in whom a febrile attack was brought on by a cold douche. Therefore we are forced to the conclusion that if under the above conditions the fever is not produced, the reason must lie in the patient himself, who has gradually become immunized against the fever-producing power of the parasites. Chilling of the surface, which has such a complex action on the blood and on the organism in general, might temporarily suspend this immunity.

These contradictory facts and their probable causes ought to be taken into consideration by those who as yet do not admit it to be a demonstrated fact for all malarial fevers that there is an intimate relationship between the sporulation of the parasites and the febrile attacks.

Fever without Any Apparent Connection with the Life Cycle of the Parasites.—In some cases of pernicious fever after the use of quinine the parasites may disappear from the blood, and in spite of this the temperature remains high for several days, and death may even occur in hyperpyrexia, the temperature running up even to 41° C. (105.8° F.). We have observed this particularly in cerebral pernicious fevers (comatose, convulsive, etc.), and it is very probable that it is connected with functional or anatomical alterations in the thermic centres produced by previous parasitic invasion. Possibly we have to do with

a fever similar in its genesis to those produced in cerebral hemorrhage or in some cases of softening, and in general to the non-infectious diseases of the nervous centres. But leaving aside these extreme cases, we are forced to admit that even in the ordinary pernicious fevers it is not possible, in the majority of cases, to establish a relation between the course of the fever and the life cycle of the parasite, even when only one generation exists in the blood. In the very grave cases it is evident that some disturbing elements must intervene, the influence of which for the most part renders unrecognizable the intimate relationship between the parasitic cycle and the course of the temperature.

Influence of Fever on the Course of the Disease.—The question has been discussed in regard to all infectious fevers whether the fever, or in a more restricted sense the elevation of temperature, is useful or not to the diseased organism; and, as we know, the replies have been various and, as a rule, based not so much on an objective examination of the facts as on the general view which those who gave them held in regard to the biological significance of the febrile process or processes. Some, regarding the fever as a reaction useful to the patient, according to a classic opinion, have gone so far as to maintain that the physician should not endeavor to combat it. We cannot enter into the general question which, with the help of experimental observations and researches, has been much discussed. We will merely recall the fact that the only way to discuss the matter profitably is to take case by case in each variety of fever. We cannot, for instance, in typhoid fever, start from a theory in regard to fever to decide whether or no antipyresis should be induced, but we must ascertain whether, in that special case, antipyresis would or would not be of use. In the case of malarial fevers we have the same problem, which is perhaps more easily studied in this than in other infections, because of the more minute knowledge which we possess as to the biology of the malarial parasite. In spite of this, in our opinion it is not possible to give a satisfactory answer to the query. It is a trite saying that the height of the fever does not as a rule correspond to the severity of the infection—this may be stated in regard to relapsing fever, some cases of septicæmia, etc. Now the fact noted above, that in grave malarial infections there may be no elevation of temperature, might be interpreted in the sense that a useful reaction is absent, but it is permitted us to assume at the most that with the insufficiency or the absence of the defensive powers of the organism there coincides an absence of temperature elevation, but nothing authorizes us to identify these powers of defence with the elevation itself of the temperature. We are also led to inquire

whether there are any indications that the febrile process, of which the high temperature is the most manifest sign, is injurious to the life of the parasite. There are some facts which would seem to give an affirmative answer; as this for instance: that during the febrile attack many mature parasites degenerate and die in the blood; and also the fact that the development and growth of the parasite occur almost entirely during the period of apyrexia. This is especially striking in the prolonged attacks of estival tertian, in which at the end of the attack we still find young parasites within the red corpuscles, while their whole development up to multiplication takes place in the few hours of apyrexia. In the third place we might quote the fact observed that sometimes a malarial patient will recover spontaneously after a grave and prolonged attack. This has been noticed chiefly in the ordinary tertian.

But opposed to these facts are others which lead us to believe that the febrile attack does not modify the development of the parasite to any great extent. For instance, we all know that for whole weeks at a time double tertians and triple quartans may follow each other with the greatest regularity. In these cases the development of the parasites, which in the quartan and simple tertian occurs almost altogether during apyrexia, takes place under the influence of repeated febrile attacks, but in spite of that it may proceed regularly without modification. This, however, does not permit us to deny that the febrile attack may be injurious to the life of the parasites at some particular moment in their existence, as, for instance, during the act of multiplication or in the phase immediately following, and not at any other time—just as quinine has a different effect upon the parasites at different stages in their life. A positive answer cannot therefore be given to this question.

Postmalarial Fever.—In addition to the febrile attacks which are connected with the life cycle of the parasites and which we have been discussing, we sometimes see a slight or marked rise in temperature, of brief or of protracted duration, after quinine has caused an entire disappearance of the parasites. This is the so-called postmalarial fever, which may last for a long while, especially after grave infections. We can give no positive information as to the pathology of this fever. Taking into consideration the fact that after the disappearance of the parasites from the blood a process is initiated by means of which the débris, so to speak, of the preceding infection is gradually removed from the system, we have proposed to call this spodogenous fever (from σποδός, cinders, ashes). But, in truth, we do not know precisely the conditions under which it occurs, nor can we say why it is observed in some cases and lacking in others.

F. Plehn and others have spoken of a postmalarial fever from quinine. We also have seen a patient who, after cerebral pernicious fever, continued for several weeks to have a febrile attack whenever he took from one to two grams of quinine, without parasites being found in the blood; neither was there albumin nor hæmoglobin in the urine. But we know too little about these quinine fevers, even from a clinical standpoint, to discuss them with profit.

The Malarial Poison is not Phlogogenic.—From what has been said so far, we can see that while the malarial parasites profoundly modify the blood crasis and produce fevers at a given period of their life cycle, they never cause acute inflammations in the real sense of the word. They never produce leucocytosis of a true inflammatory type, nor, consequently, inflammations with exudation of leucocytes. This was clearly shown some time ago by Baccelli, from clinical observations; and we may add that pathological anatomy and the knowledge we possess as to the pathogenic action of the parasites, described above, absolutely confirm these observations. Some more recent writers (as Laveran) speak of malarial pneumonia, endocarditis, etc., but we can positively assert from the result of numerous autopsies and bacteriological researches that in all these inflammatory processes we always find a secondary infection by pyogenous schizomycetes or by the pneumonia diplococcus. As secondary effects of the malarial infection, however, we may have certain alterations in various viscera like those of chronic inflammatory processes; as hepatitis, nephritis, etc. These lesions, however, as pathological anatomy teaches us, should be considered as secondary to the degenerative or necrotic lesions which are produced in the parenchyma of the various organs by the parasitic invasion. Even the acute nephritis occurring in malaria may be held to be an acute degenerative lesion of the renal parenchyma, probably of toxic origin; and the acute enteritis and colitis of malarial origin are, in our opinion, secondary to necrosis of the epithelium of the mucosa determined by the parasitic invasion, and to the growth of the germs which inhabit the intestinal tract in the portions of mucosa thus altered.

The Urine in Malaria.

Many investigators have for a long time studied intermittent malarial fevers in order to ascertain what are the alterations in metabolism secondary to a febrile process of short duration. From this point of view malarial fevers offer a fine field for research, so that a study of the urine in malaria is of importance in regard not only to

the pathology of this infection, but also to the general pathology of infective fevers.

The behavior of the urinary secretion during the attacks, in the interval between attacks, and during convalescence, the modifications in its amount, the relative proportion of the various constituents, etc., have been the subjects of much research. We shall base our remarks chiefly on the work of Rem-Picci, who has long interested himself in this subject at the medical clinic of the University of Rome.

Amount.—If the urine of twenty-four hours be collected from a malarial patient during the course of an acute infection, we do not, as a rule, find marked variations from the normal as to quantity. In tertian and quartan fevers, however, the amount is more than usual, while in estival infections it is often less—that is to say, the estival infections in this respect more nearly approach the condition of continued fevers. During the febrile attack, as a rule, there is an increase in the amount of urine; this has been noted by various authorities, and is explained by Riegel as due to increased vascular tension during the chill, and to a diminution in the loss of water from the cutaneous surfaces. But even for a time immediately preceding the attack and following it the amount of urine may be increased; the minimum is obtained in the interval of apyrexia between the attacks. There are exceptional cases in which the greatest amount is found after the fever, and not during the attack.

The *specific gravity* of the urine passed during the attack is often increased, however great the amount may be. This is due to the absolute increase of the salts and nitrogen eliminated. Even in the polyuria of the first hours of the attack (urine passed after the chill) the specific gravity may be high.

After the fever has been checked by quinine or has ceased spontaneously, we often observe what has been called the *polyuria of convalescence.* Mossé states that this polyuria occurs from the third to the sixth day after the cessation of the fever, and disappears either rapidly or by degrees; he observed it in about a third of his cases (eleven out of thirty-six); Rem-Picci found polyuria in sixty out of one hundred and fifty cases, but it is likely that it occurs even more often, because it may not begin until several days after the cessation of the fever, and many patients have already left the hospital and are beyond observation by that time. It may, however, appear immediately after the cessation of the fever, or even during the last attacks. Its duration may be of several days, or it may be protracted as long as a month. The amount is usually from two to three litres (four to six pints), rarely more. Rem-Picci has never seen it go higher than six litres in the twenty-four hours. It is found in both

primary cases and relapses, after severe and after slight attacks, in the weak and in well-nourished persons. It is worthy of note that it is more often observed in tertian and quartan than in summer-autumn fevers, a difference which seems to be owing to the febrile species, and not to accidental circumstances or to the different seasons in which these fevers predominate. The specific gravity of the urine is increased relatively to the amount, owing to the abundant elimination of solid substances. The greatest elimination occurs during the night, a fact that is usually observed also in malarial patients who have not polyuria. As to the significance of the polyuria in convalescence, it may be stated that it does not depend upon alimentation. Nor can it be regarded as a phenomenon of recovery, because it is often absent in cases of spontaneous cure, and sometimes present during the course of the fever. But according to Rem-Picci, it is an eliminative polyuria, that is to say, it is due to the attempt of the organism to rid itself of the débris of the tissues decomposed during the fever but remaining in the organism.

Much rarer than, and differing from, this polyuria of convalescence is the *chronic polyuria*, which occurs in chronic malarial patients and cachectics. As in simple polyuria, the urine is of a low specific gravity, ranging from 1.005, even when the amount is above 4,000 c.c. (eight pints) in the twenty-four hours. The amount of urine may be very great. It would seem that in some cases the polyuria of convalescence passes into simple chronic polyuria, in spite of the difference between the two varieties.

Physical Properties.—The urine of malarial patients is usually highly colored, especially during the attack; it often becomes turbid after it is passed, and if left standing throws down a sediment composed sometimes of urates and sometimes of phosphates. In post-malarial polyuria the urine is not so pale as is that of dilute urine in general; often it is of a golden yellow color, and gives the reaction of urobilin.

Urea.—It is known that malarial fevers have served as a basis, so to speak, for the establishment of the fact of the increased elimination of nitrogen in fevers, and of the increased consumption (in general) of albumin. Many researches have been made in this direction.

The amount of nitrogen eliminated by malarial fever patients is nearly always above the normal, but is not excessive. We cannot state it to be the rule that there is greater elimination of nitrogen in robust patients recently infected than in those suffering from relapses, and in the anæmic. This might be supposed to be the case, from what is known as to the behavior of anæmia, but evidently the total amount of the nitrogen is influenced by so many factors, such as alimenta-

tion, absorption, etc., that we cannot expect that there should be an exact and constant relation between the facts in question.

As to the phenomena induced by the febrile attack, we know that during the attack there is usually an increased elimination of nitrogen, which is not, however, constant; there are cases indeed in which the least elimination occurs during the fever. The first is most marked in quartan fevers; the second, it would appear, in some fevers with short intervals of apyrexia. For instance, in one case of quartan, in which the fever relapsed as a quotidian (triple quartan), Rem-Picci found during the quotidian a greater elimination of nitrogen in the apyrexia than during the fever, while during the regular quartan the increase of nitrogen coincided with the febrile attack.

As a rule, the greatest amount of nitrogen is eliminated in the first hours of the fever; probably the increased blood pressure and the great destruction of red corpuscles at this time are the causal factors. In this the observations of the various writers agree. Exceptionally there is an increase before the fever, the so-called prefebrile increase. The lowest excretion of nitrogen, but not below the normal, is usually found after the attack. But in the cases to which we have referred, in which there was slight elimination during the fever, the maximum occurred afterwards. We may infer from this that the urea formed during the fever is eliminated later only, because it is well known that even physiologically the formation of urea does not always coincide with its elimination.

Sidney Ringer has called attention to the fact that during convalescence we may have a greater elimination of nitrogen in the time corresponding to the expected febrile attack, even when this does not take place. This has been confirmed by others, and in one case by Rem-Picci.

It seems to us that from the facts given above we may draw the conclusion that the elimination of nitrogen cannot be held to be chiefly and primarily in direct relation to the number of red corpuscles destroyed; because if this were the case, there should be a great difference in the effects of a febrile attack according to whether it is primary or a relapse, the destruction of red cells being much greater in the former case. Neither can we consider it to be intimately connected with the height of the temperature. It is not the elevation of the temperature, but the infection, that increases the tissue waste to which the increase of nitrogen is due.

Uric Acid.—While there have been many researches as to the variations in the amount of nitrogen, those of Rem-Picci are about the only accurate ones which have been made in regard to uric acid. The conclusion drawn from his investigations is that no special and

constant law can be laid down as to the excretion of uric acid during the febrile attack. We can only say, and even then only approximately, that the proportion of uric acid usually falls before the fever, and that it is rather high during the attack. But the attack has no positive or constant effect upon the elimination of uric acid, which proves the independence of the formation of uric acid and that of nitrogen, the latter, as we have shown, obeying special laws. It is well known that the urine of malarial patients is often turbid from undissolved urates, and that it often throws down a brick-dust deposit, but this may be merely a question of the solubility of the uric acid in this particular urine, and not of an increased production.

Finally it may be noted that in spite of the presence of an enlarged spleen, which is so constant and characteristic in malaria, there is no constant increase in the excretion of uric acid, such as we find in other diseases associated with an enlarged spleen.

Chlorides.—The researches as to the elimination of the chlorides have been more extensive, but rather contradictory in their results. Rem-Picci and Caccini have studied thirteen cases of tertian and quartan fever, primary and relapsed, some cured by quinine, some recovering spontaneously. From their researches we find that in the first hours of the attack there is a marked increase in the amount of urine and of the chlorides, and then follows a gradual diminution which usually attains its maximum when the fever falls; immediately after the fever the amount of chlorides excreted continues subnormal for about twenty-four hours, but it is not marked or constant. We should judge from the results of the researches that these variations do not depend upon alimentation, but are in intimate relation with the phenomena characterizing the febrile attack.

Thus we may regard the increased elimination of chlorides, especially at the beginning of the attack, as related to various factors, such as the destruction of the red cells, the increased blood pressure during the chill whence also the increase in the amount of urine, the lavage of the tissues, etc., and in general the greater destruction of the tissues themselves. The diminution immediately following the attack may be explained by assuming that after the increased elimination due to the attack, the blood retains the chlorides introduced into it. We may further suppose that the blood must retain chlorides while it is recovering from the loss of the red cells.

During convalescence we sometimes notice a marked elimination of sodium chloride with polyuria and azoturia. For the most part there is a certain relationship between the volume of the urine and the amount of chlorides and of nitrogen.

Phosphates.—There have been many researches into the matter of

the elimination of phosphates during febrile diseases. In diseases with a continued fever there is usually a decrease, though it is not certain whether this is owing to the fever or to the changed diet. Many authorities hold to the latter view. Vogel, who has made an analysis of the phosphates in the urine in more than a thousand cases of various diseases, regards it as a mere question of alimentation.

As to malarial fevers, Rosenstein, who studied a case of quartan during two attacks, found a diminution of phosphates during the fever, which he attributed to the lack of food. Gee, who studied two cases of tertian, giving food during the fever in order to exclude the question of alimentation, found that the phosphoric acid in the urine diminished at the beginning of the febrile attack, was reduced to a minimum during the acme of the attack, rose towards the end of it, and amounted to two or three times the normal almost the instant the fever fell. Freund in two cases of malaria noted a diminution of phosphorus, even to complete disappearance from the urine during the fever, and found that when he gave the phosphate of sodium by the mouth there was still a diminution of phosphoric acid in the urine. The results of the more recent researches of Rem-Picci and Bernasconi, which were much more extensive and were surrounded by all necessary precautions, agree with these. These observers studied five cases of quartan, three of tertian, two of double tertian, two of malarial cachexia, and three of recently cured infection. Their conclusions were that, in malarial fevers the amount of phosphoric acid eliminated in the twenty-four hours is often increased above normal. In their cases alimentation was usually abundant.

Almost immediately after the temperature begins to rise there is always a marked diminution of phosphates in the urine, while the quantity of urine is usually increased. This fact is the more remarkable, because physiologically a greater amount of urine means a greater elimination of phosphorus. This diminution in the phosphates is not in proportion to the duration and the degree of the fever and is independent of alimentation; in fact, it occurs even when the attack comes upon the patient in full digestion, so that the food ingested is quite equal in amount to that taken on the days of apyrexia. Moreover, during the fever there is usually an increase in the elimination of the chlorides and of nitrogen. Now, if all depended upon defective alimentation, these substances should be the first to be influenced. Besides this, if we administer sodium phosphate by the mouth or inject it subcutaneously before the fever, it is not excreted in the urine during the fever.

The diminution which has occurred during the fever is usually

followed, upon the subsidence of the latter, by an increased elimination of phosphoric acid. This postfebrile discharge of phosphates usually occurs immediately after the cessation of the fever, and lasts for several hours, usually completely compensating for the febrile diminution.

When the infection has been cut short by quinine, we often, but not always, find a phosphaturia which is sometimes most marked and accompanied by more or less profuse polyuria. Everything leads us to suppose that during the fever there is a retention of phosphates in the organism, and probably of other substances as well, since the amount of the urine may be much increased, with a high specific gravity. This abundant elimination of accumulated matters may be injurious to the kidneys, as shown by the cases of postmalarial albuminuria in which the albumin is transiently present during the polyuria and the phosphaturia.

In a further series of researches, Rem-Picci took up the question of the elimination of phosphates injected subcutaneously during the apyrexia and during the stage of fever, and he came to the conclusion that during the fever the kidneys only partially allow the passage of phosphoric acid, no matter in what combination it is found.

Sodium and Potassium.—It is well known that the elimination of these two alkalies in the urine is of great value in the study of the physiology and pathology of metabolism, because as these two substances exist in different proportions in the various tissues and fluids of the body, their elimination may approximately indicate what kind of tissue has been destroyed in a given period of time. Normally more sodium than potassium is eliminated (1.5–2 of sodium to 1 of potassium). On the other hand potassium is more abundant than sodium in those conditions in which the body destroys its own tissues, such as protracted fevers and fasting.

Salkowski has demonstrated the way in which the elimination of these two bases occurs in febrile diseases, especially in fevers of long duration. As to malarial fevers, we obtain the clearest results in those of a regular type. Rem-Picci has ascertained the following facts:

A patient suffering from regular attacks of malarial fever nearly always eliminates more sodium than potassium in the course of twenty-four hours, as he does normally; yet the proportion is not the same as under normal conditions, there being a marked tendency to an equalization in the amounts of the two bases, hence there is a relative increase in the amount of potassium. During convalescence both increase, and, what is of great interest, the potassium takes the lead, so that in some cases there is an inversion of the normal ratio between them.

As to the influence of the febrile attack, we find that the maximum elimination of sodium and potassium occurs during the hours of fever, at which time the amount of urine is also greatest; during apyrexia the values are lowered and may even go below normal. In the intervals the elimination of potassium is greater than that of sodium, relatively and sometimes absolutely, as it is in convalescence.

Cases of estival fever do not give such regular results, yet they do not contradict the laws given above. The lack of constancy in the results is due to the fact that in these fevers it is not possible to obtain prolonged observations and regular alimentation, because of the brevity of the periods of apyrexia and the severity of the attacks.

The difference between the elimination of sodium and potassium in the urine of malarial patients and that in infections marked by continued fevers (Salkowski's data) is explained by the fact that in malaria alimentation is abundant. In fact, in the grave cases of almost continuous malarial fever in which little food is taken, the alkaline bases behave in the manner described by Salkowski; that is to say, the excretion of both is low, but that of potassium is at a constantly higher level than that of sodium.

The excess in the excretion of sodium during the attacks of regular fevers is probably peculiar to malaria, and is possibly in relation with the abundant diuresis. The contemporary excess of potassium can be due only to the febrile decomposition of the tissues, to which is added that of the red corpuscles, which, as we know, are rich in potassium.

While during convalescence from the continued fevers there is a return to the normal ratio in the excretion of sodium and potassium, and not only that, but more sodium is eliminated than is ingested (so that some authorities believe that there is retention of it during the fever) and less potassium than is introduced (it being evidently retained to assist in the repair of the wasted tissues), in convalescence from malarial fevers we do not have the same conditions. There is an absolute increase of both bases, but proportionately more potassium than sodium, so that an inversion of the normal ratio is almost the rule. This is the exact opposite of what occurs in other infections. The increase in both bases can be explained only by the same theory that we hold in regard to postmalarial phosphaturia and hyperchloriduria, that is to say, by the retention of sodium and potassium during the febrile period. We can exclude all dependence upon alimentation, not even the increase in potassium being due to it. It is to be accounted for rather by the elimination of débris, which from the time of the attack has remained and infiltrated the tissues; such are the necrotic elements (leucocytes, etc.) which are found in the

viscera in great number after a series of febrile attacks, and the removal of which precedes the regeneration of the tissues.

In discussing the polyuria of convalescence we stated that in this the greatest elimination of water (which may sometimes be three times as great as that passed in the daytime) and of solid matters occurs with a surprising regularity during the night hours. We therefore have the inverted type of Quincke in the urinary secretion, as occurs in cardiac and nephritic patients; moreover, in this nocturnal urine the normal ratio, sodium > potassium, is usually inverted to potassium > sodium, just as in fasting and in febrile states. Nocturnal repose, therefore, appears to be favorable to the elimination of the substances derived from the residua of febrile tissue waste, residua which are stagnating, so to speak, in the tissues themselves and are expelled by the eliminative polyuria of convalescence.

Iron.—We know that the urine of fever patients usually contains more iron than that of the healthy. Now, according to the researches of Colasanti and Jacoangeli, the urine of malarial fever patients contains on an average more iron than that of persons suffering from other fevers. The amount of iron eliminated is greater after than during the attack, and continues for some days after the disappearance of the parasites from the blood. It is in proportion to the gravity and duration of the disease, and is greater in primary infections than in relapses. Generally speaking, according to these investigators, every increase in the elimination of iron corresponds to a diminution in the amount of hæmoglobin.

The same writers also determined the amount of iron in the fæces in five malarial patients, by Hamburger's method. Comparing this with the amount of iron in the fæces of a healthy individual who was living under similar conditions, they found that iron is perceptibly increased in cases of a certain gravity, and the increase is always greater than that in the urine, but goes on *pari passu* with the latter. This iron found in the intestinal tract is derived not so much from the various digestive secretions as from the metamorphoses which the coloring matter of the blood undergoes in the liver in the formation of pigment. This leads to the belief that the increase of iron in the fæces of malarial patients is in relation with the polycholia, which is known to be excessive in grave infections.

Toxicity of the Urine.—Many authorities have found an increase in the toxicity of the urine in malaria, and have attributed various significations to it. Roque and Lemoine studied three cases; one of typical tertian, in which there was an increased toxicity of the urine after the attack, and two of pernicious fever, in which there was

marked increase after the administration of quinine. From these data they concluded that the malarial parasites manufacture in the blood a large quantity of toxic products which are in great measure eliminated by the kidneys, chiefly after the attack; and that quinine sulphate acts by favoring or increasing this elimination. It seems to us that the data are not sufficient to justify such conclusions. If there is so great a production of toxic substances through the action of the parasites, we cannot understand why Celli was unable to demonstrate an abnormal toxicity of the serum at various periods of the febrile process.

Bottazzi and Pensuti studied this subject in ten cases of malarial fever, some of ordinary tertian, and the others of estival fever. They found that the urine passed during apyrexia was more toxic than that eliminated during the fever (thus far being in accordance with the above-mentioned authorities) and that it was more toxic than normal urine. This toxicity was usually increased by a succession of febrile attacks, but there were cases in which the urotoxic coefficient rose and fell irregularly. According to these writers this increased toxicity can be explained by the changed proportions in the usual constituents, without the necessity of invoking the action of special toxic substances. They incline to attribute a large part of this increase in toxicity of the non-febrile urine to the increased elimination of urobilin.

Abnormal Substances.—Among the abnormal substances which may be found in the urine of malarial patients, we will first mention *serum albumin*, which has been found in varying proportions by different observers. In our experience, for instance, albuminuria in malaria is rare, while Marchoux found it in nearly all his cases. It would appear, therefore, that the frequency of albuminuria varies in the different malarial regions. For a description of the various kinds of albuminuria which may occur in malaria the reader is referred to the sections on Sequelæ and Complications. *Nucleoalbumin* has been found several times by Mannaberg in the urine passed during or after a febrile attack. The presence of *peptone* has been demonstrated by Bottazzi and by Pensuti. As to *glycosuria* see the sections on Sequelæ and Complications. *Urobilin* is often found in large amount. The quantity of *indican* is frequently increased. The *diazo reaction* of Ehrlich was found in 5.5 per cent. of the cases studied by Thayer and Hewetson, and in some cases also by Mannaberg and by ourselves. In some cases, rare in our experience at least, the febrile attack is accompanied by a more or less severe *hæmaturia*. In an estivoautumnal patient with severe hæmaturia during the attacks, recently under observation in the hospital of Santo Spirito, De Rossi

found in the sediment of the urine red corpuscles containing parasites.

For a description of the characteristics of the urine in *hæmoglobinuria* see the section on that subject.

PATHOLOGICAL ANATOMY.

The pathological anatomy of malaria is so closely related to the biology of the parasite that without a knowledge of the latter no serious study of the former will be possible. Hence the relatively slight importance of the anatomico-pathological observations made before the etiological studies, because to the alterations actually due to malaria were joined others of varied etiology, or those really due to malaria and constituting the most important part of them were not recognized as such.

The studies made in Rome by Bignami and Guarnieri, and others elsewhere before and after them (Laveran, Councilman and Abbott, Bastianelli, Dock, Barker, Monti, and others), but especially the former, not only brought in evidence the anatomico-pathological alterations due exclusively to malaria, and their pathogenesis, but they also defined them most exactly, throwing out many inaccuracies proceeding from insufficient observations and many hypothetical interpretations. For this reason we shall take as our guide in the discussion of the pathological anatomy of this infection the studies made by Bignami and Guarnieri, adding to them the later observations made by ourselves and others.

We may profitably premise our study with a few definite statements: The malarial infection develops in the blood; here only, and chiefly within the red corpuscles, can the parasite live. From this it follows that the parasite invades the red corpuscles and nourishes itself at their expense, transforming the coloring matter of the corpuscles into black pigment (which, after the multiplication or the destruction of the parasite, is incorporated into the white cells) or otherwise injuring the red corpuscles. In consequence of this infection of the blood we find, in addition to the destruction of the cells, a production of the detritus of the red corpuscles and of the parasites, the presence of pigmented white cells, and the penetration of erythrocytes containing parasites and of leucocytes containing pigment into the capillaries of all the organs. It can be understood from this primary localization of the infection how the principal changes must be found in the hæmatopoietic organs in addition to the blood, and how alterations are to be encountered in all the organs and in all the tissues.

Acute Malaria.

We will now review in their order the various alterations which are seen post mortem in the bodies of those dead of an acute malarial infection. External examination of the body shows a pale brownish color of the skin—an earthy discoloration. The conditions of general nutrition will be found to be various according to the duration of the disease.

Brain.—In cases of cerebral pernicious fever the leptomeninges are often intensely hyperæmic, as is also the cerebral substance. Hyperæmia alone will not permit us to make the diagnosis of pernicious malaria, but this can be readily made when to the hyperæmia is conjoined melanosis. When this is present, the cortex and the gray substance of the ganglia and crura assume a brownish-red or blackish color. Such variations of color are explained by the results of microscopical examination. In cases which are not at all exceptional we find in the nervous centres punctiform hemorrhages which are almost always in the white substance. These hemorrhages are sometimes few in number and collected more in one part than in another, sometimes so numerous, so close, and occupying such an extensive area as to give a rosy tint to the white substance of the brain. When the hemorrhages are circumscribed, their seat is variable; now in the white substance of the brain mantle, now in the trunk, as, for example, in one of the internal capsules, and now in the cerebellum. In the latter the hemorrhages occur also in the gray substance. It is to be noted that in some cases of pernicious fever (choleraic, algid forms, etc.) when the infection has lasted a number of days, or in the relapses, the brain and its membranes may be found quite anæmic.

The microscopical examination may be made of a fresh specimen by compressing a very minute portion between the slide and cover-glasses; or in preparations made by tearing out a little particle of the cerebral cortex, according to Bignami's method, and then staining it; or in colored sections. Whichever of these methods is employed we see that in the cerebral capillaries there are parasites generally enclosed in the red globules with which the lumen of the capillaries is filled. The parasites are encountered in the different phases of their cycle of existence or one of these phases predominates, or one even exists alone. Thus we may have (*a*) cases in which all or nearly all the endoglobular parasites are without pigment, and then the brain is intensely hyperæmic but not pigmented; (*b*) cases in which the parasites contain but little pigment, and then the brain

is hyperæmic and slightly pigmented; (*c*) cases finally in which the parasites contain much pigment and are very near to sporulation or already in that stage, and then the brain is intensely pigmented. But, as we have said, we frequently find the parasites in all their stages of development, so that it is sometimes possible to demonstrate in a single capillary the entire cycle of the parasite's existence. The number of parasites is sometimes so great that it is difficult to find a single normal erythrocyte. Except in very grave cases in which almost all the red blood cells are infected, the arterioles and the small veins are less rich in parasites than the capillaries. In very exceptional cases we may follow the entire evolution of the parasite from the small endoglobular amœba to the stage of fission without pigment having been formed (hæmamœba immaculata). But these cases are, as we have just said, very exceptional and many who have devoted much time to the study of malaria have never seen them. It is more common to find pigmented and non-pigmented parasites in the various phases of their existence, although one of the latter will prevail over the others. Then the adult pigmented forms and the sporulations occupy by preference the capillaries, while in the veinlets and the arterioles we see for the most part the young forms, and these grouped especially along the vessel walls. In some cases we do not find a large number of parasites, but we see the traces of a progressive parasitic invasion in the little collections of pigment, in the swollen and pigmented endothelial cells, and in the pigmented leucocytes.

The changes in the endothelium of the capillaries deserve special mention. Many of these elements are swollen and in a state of fatty degeneration; in transverse sections of the capillaries their lumen is seen narrowed and even closed by the swollen endothelial cells. This alteration is encountered more frequently in the pigmented cells, but is seen also in those which are not pigmented. In the endothelia, in addition to the pigment may be seen parasites and sometimes numerous spores.

In cases in which sporulation abounds we may find accumulations of spores and masses of pigment almost occluding the lumen of the capillaries—a true parasitic thrombosis.

In addition to the changes in the endothelium lesions of the nerve cells in the nuclei and protoplasm, and particularly in the cells of some of the nuclei of the bulb, were found by Marchiafava in a case of pernicious fever with bulbar symptoms. Recently Monti, studying by Golgi's method the condition of the elements in the central nervous system in cases of pernicious fever with cerebral symptoms, made some observations of much importance in the interpretation of

malarial and postmalarial nervous symptoms. It is not common, in cases of pernicious fever with cerebral symptoms, to find any evident or notable changes in the nerve cells when examined by Nissl's method. We would note that for many years, even before this method became so extensively employed in laboratories in the study of the pathological histology of the nerve cells, it was the custom in our laboratory to use fixation in alcohol or in sublimate, and to stain with methylene blue or Bismarck brown and with magenta or other aniline colors, in the study of the malarial lesions of the nerve centres, for the reason that with this method we could not only study the nerve cells but also observe very clearly the parasites and the alterations in the vessels. It was due now to the relative rarity of evident nerve changes that we did not long ago insist upon them more particularly. These changes may involve the cell body as well as the nucleus. Thus in a case of comatose pernicious fever in which the coma was very protracted, there was found post mortem an enormous parasitic invasion of all the viscera, but especially of the brain, the vessels of which were literally stuffed with parasites containing central masses of pigment or in process of fission, and the resulting melanosis was of an intensity that is rather rarely encountered; the walls even of the small vessels were notably altered by the pigmentation and the swelling of the endothelial cells. The changes in the protoplasm of the nerve cells could be distinctly seen in preparations stained with methylene blue or magenta, especially in the large and small pyramidal cells. In some of these elements the protoplasm presented itself as finely granular through the disappearance of the chromatic bodies of Nissl, and had the aspect described by many authors as the result of chromatolysis; this may be total or partial. In other cells the changes are more grave and the protoplasm appears as if rarefied and disaggregated, presenting a spongy appearance, or as if split up into drops. In these elements, notwithstanding the grave alterations of the protoplasm, the nucleus may present a normal appearance.

More important than this chromatolysis, which is a change noted by numerous observers in many infections and intoxications, are the nuclear alterations. The altered nuclei may, in the pyramidal cells, present a varied aspect. In some cells the nuclear membrane and the nucleolus are not visible, and the strongly colored nuclear chromatin is divided into little more or less regular masses, so that in some cases the nucleus has a mulberry-like appearance. In other cases the nuclear membrane persists, and the little chromatic masses are grouped in the centre separated from the membrane by a clear zone. More rarely we see the nucleus pale in consequence of the disappear-

ance of both the nucleolus and the nuclear chromatin. Very rarely the nucleus is seen displaced towards the periphery of the cell and as if emerging from it. These nuclear alterations, in some of the elements, by reason of the increase of the nuclear chromatin and of the disappearance of the membrane, may at first sight be taken for the initial stage of karyokinesis; but they are to be interpreted, taking into account the entire histological picture, as due to phenomena of karyolysis and of karyorexia, or as necrobiotic figures. The changes described are found irregularly disseminated in the cortex, although the parasitic invasion is total, and they are not found in the same case in the bulbar nuclei, contrary to what has been seen in a case of pernicious fever with bulbar symptoms. Indeed, even the bulbar vessels in the case referred to were filled with the parasites just as those of the cerebral cortex.

If we recall the nuclear changes in the nerve cells which have been described as occurring in experimental ischæmia, we cannot fail to note the resemblance to those just mentioned as found in malaria; and so we are led to believe that, without excluding the action of hypothetical toxic substances produced by the parasites, we may perhaps find a sufficient explanation of these facts in remembering that the filling of the brain with red blood globules containing these parasites acts in the same way as a temporary ischæmia. We shall have to return to the alterations just described when we seek to give an explanation not only of the cerebral symptoms of pernicious fever but also of the nervous symptoms of more chronic course which may follow a grave malarial infection.

The same alterations which we have described as occurring in the vessels of the brain are encountered also in those of the pia mater, in which sometimes all the red corpuscles are infected by parasites. We find in these more frequently than in the cerebral vessels pigmented phagocytes, often very large, and sometimes in such numbers as to occlude the vascular lumen.

The description just given applies to cases of pernicious cerebral fever, especially the comatose variety. In the other forms of pernicious fever, as the choleraic, we usually find few parasite-infected red corpuscles, and the changes in the endothelium are lacking.

A microscopical examination of the punctiform hemorrhages demonstrates that they are situated around the blood-vessels, the endothelium of which is altered in the manner already described; that the extravasated red corpuscles are normal, even when many parasite-infected corpuscles are found within the blood-vessels. Bastianelli and Bignami, who have given much attention to the pathogenesis of these punctiform hemorrhages, hold that they are due to diapedesis through

the altered walls of the arterioles, and occur nearly always in the white substance, because in this situation the capillary network is less dense, and the lumen of the vessels is smaller than in the gray matter, a fact which favors stasis up to the point of thrombosis.

In the *spinal cord* the same changes are found as in the brain.

Retina.—A study of the changes occuring in this tissue is essential to an understanding of the transitory and permanent alterations in the visual function, of which we shall speak presently. Macroscopically the only change perceptible consists in the punctiform hemorrhages which are somewhat numerous.

Guarnieri has recently made a microscopical study of the retinal changes which occur in malaria. The capillaries are injected with parasite-infected red corpuscles, which are deformed and have lost the power of being stained by eosin; the veins are dilated and deformed, with œdema of the lymphatic sheath, and contain (sometimes in such numbers as to occlude the lumen) melaniferous phagocytes and parasite-infected corpuscles, which are also found in even greater abundance in the veins of the choroid. The hemorrhages involve the external layers of the retina, especially the external plexiform layer.

In the retinal hemorrhages also, the extravasated corpuscles contain no parasites. As to the distribution of the amœba-containing corpuscles in the retinal blood-vessels, we find that the capillaries contain red corpuscles with parasites at an advanced stage of development, while in the larger blood-vessels the corpuscles with adult parasites are found clinging to the vessel wall, in the same way that white cells cling to the endothelial layer of the veins in an inflamed area. Phagocytosis does not occur in the endothelium of the blood-vessels of the retina.

In the nervous apparatus of the retina we find changes secondary to the hemorrhages, as well as to the accompanying stasis and œdema. Up to the present time, these changes have appeared to consist in a confused arrangement of the cells of the internal granular layer.

Heart.—In cases of recent infection, the heart, and more especially the right ventricle, contains coagula of red corpuscles and fibrin, and brownish-red disintegrated blood. A microscopical examination of the latter shows a variable number of parasite-infected red corpuscles, pigmented leucocytes, and endothelial cells, some of which contain pigment. Our observations have demonstrated the occasional presence of subpericardial hemorrhages and the flaccidity and dilatation of the walls of the heart, especially on the right side, a fact which may often be noted clinically.

Lungs.—In relation to the duration of the disease, we find hypostatic congestion, œdema, hemorrhages, and areas of hypostatic pneu-

monia. A microscopical examination usually shows the following alterations: In the capillary network are seen large pigmented or globuliferous phagocytes, with a large vesicular nucleus, some in a state of degeneration. The polynucleated white cells with pigment masses are rare. A transverse section of the veins may show an accumulation of pigmented macrocytes clinging to the vessel walls, sometimes in such numbers as to take up a third of the lumen.

The parasitic forms correspond to those found in the brain, and are usually in all the phases of existence with the special predominance of one. It is very evident that the parasites have a tendency to accumulate in the arterioles and capillaries, while they are found in decidedly lessened number in the veins where the pigmented and globuliferous phagocytes abound. This fact proves that in malaria the lungs are the seat of a phagocytic process.

In cases of bronchopneumonia which occur somewhat frequently in pernicious infections of protracted course, we find that the exudate is chiefly cellular and hemorrhagic, and to a small extent fibrinous; that this exudate contains the ordinary polynucleated leucocytes, while the capillaries of the septa are filled with large pigmented and globuliferous phagocytes. This lack of diapedesis on the part of the pigmented leucocytes appears to be in contradiction to what occurs in experimental inflammations, when finely granular substances are introduced into the blood, and found again in the extravasated leucocytes. But if we consider that in the phagocytes containing pigment and parasite-infected red corpuscles we find the signs of retrogressive changes, we shall easily see that in all probability the amœboid movements, by means of which diapedesis is effected, are either diminished or altogether extinguished according to the degree of the necrobiosis. Further, the large uninuclear leucocytes which act like phagocytes in malarial blood, are incapable of diapedesis.

The ordinary diplococcus of pneumonia has been found in all the cases of bronchopneumonia or of lobar pneumonia occurring during the course of pernicious infection which have been studied bacteriologically up to the present time. Hence there must be a double infection.

Spleen.—This organ is always increased in size. The capsule is tense, the parenchyma chocolate colored or black, very much softened and sometimes diffluent. In such a condition of the organ it is easily lacerated if removed without great care, especially when there are adhesions. This condition may also account for spontaneous rupture of the spleen during life. It is difficult to distinguish the various parts of the pulp, and even the Malpighian corpuscles are not always recognizable.

For a microscopical examination of the spleen we may use fresh preparations or sections taken after hardening, etc. In the fresh preparations we see red corpuscles containing parasites in the various stages of their life cycle, leucocytes, usually large and containing pigment in grains or blocks, parasite-infected red cells, free parasites, and fragments of red corpuscles. Some of these cells, which are often of enormous size, may show signs of retrogressive changes in the nucleus and the protoplasm, while others are most evidently necrotic.

On examination of a hardened section we usually find the trabeculæ of the pulp invaded by an immense number of red corpuscles, which separate the cells of the pulp itself, and which usually contain parasites in the various phases of their existence with predominance now of one and now of another phase. If the infection has lasted several days, crescent bodies are rarely absent, even when they are not to be found in the circulating blood. In addition to the parasite-infected red corpuscles, there are the large phagocytes which have already been described, pigmented, parasite-infected, some of which attain a gigantic size and contain in addition to pigment brassy bodies with parasites as well as fragments of brassy bodies, free parasites, and even sporulating bodies. As we have already mentioned, degenerative changes extending even to necrosis are found in these cells.

Pigmented, polynucleated cells are rare. The small mononucleated cells are non-pigmented. The Malpighian follicles, whose cells are not pigmented, are in strong contrast with the intensely pigmented trabeculæ of the pulp. While the capillaries are usually filled with red amœba-containing corpuscles (as can best be seen in an enlarged spleen), the splenic veins contain but few of them, and these few cling to the weakened walls; but they do hold many large phagocytes rich in pigment and fragmented red corpuscles, also clinging to the vessel walls. Many of these macrophagi are found in the blood of the splenic veins. Among the cells of the pulp and of the Malpighian follicles, many are found undergoing karyokinesis.

The condition revealed by the microscope explains the macroscopic characteristics observed, namely, the softening of the splenic pulp by hyperæmia and the acute œdema, the dark red or blackish coloration due to the unusual accumulation of pigment, the tension of the capsule, etc. What is of still greater importance, it also shows how the blood is purified from parasites, namely, by their inclusion within the macrophagi at every stage of life from that of the non-pigmented amœba within brassy bodies to the sporulated forms.

Liver.—The liver is increased in size. The cut surface is smooth,

shining, and of a brownish-red or even a slaty color. The consistency of the organ is diminished. Much blood oozes from the cut surface of the organ.

Microscopically we find within the capillaries an accumulation of pigmented macrophagi which in certain sections appear to occlude the lumen, but as a rule there are few parasites. The endothelial cells of the capillaries are swollen and project into the lumen, while their nuclei are altered, and their protoplasm contains balls or large lumps of pigment and round hyaline bodies which are supposed to be dead plasmodia. Free endothelial cells are sometimes found in the lumen of the blood-vessels. Pigmentation is also found in the perivascular stellate cells of Kupfer. It is, however, never found in the hepatic cells, which present lesions of more or less gravity, some being atrophied, some showing degenerative processes of the nucleus, and others being necrotic. Necrosis is found in isolated cells, or in cells gathered together in little groups. In addition to these changes we find cells undergoing karyokinesis which belong either to the glandular cells or to the cells of Kupfer, and which represent a regenerative process.

The necrosis is partial and limited to certain areas. In one case of comatose pernicious fever with icterus, in which, after the fever had yielded to quinine, coma persisted and ended in death, there was found a flaccid icteric liver, not diminished in size, and while the gall bladder was distended the bile ducts were pervious. A microscopical examination showed extensive necrosis of all the hepatic parenchyma such as we find in grave icterus of rapid development. In addition, there was extensive fatty degeneration of the kidneys, heart, etc. It is worthy of note that even after death there were found large numbers of parasites in the various stages of their existence, both in the circulating blood and in the organs, especially in the brain.

We have said that the hepatic cells do not contain black pigment, but another form of pigmentation, the *ochraceous*, is found in their protoplasm. This proceeds from a variable number of yellowish, shining dots, which behave towards the various staining agents like the fragments of hæmoglobin included within the globuliferous cells. Some of these little masses are the size of a small red corpuscle, and show crenated borders, as if they were shrivelled. A fresh preparation of scrapings from the liver shows this yellow coloring-matter quite distinctly; we can see the individual hepatic cells much swollen and loaded with yellow granules. In hardened sections we see that the pigmentation is more intense around the central vein of the lobule, gradually diminishing towards the periphery. As to the origin of this pigmentation, the most plausible explanation is that it is

due to a deposit of coloring-matter derived from necrosed red corpuscles (brassy bodies) within the hepatic cells. In relation to the above condition, we have the polycholia and the hæmohepatogenous icterus found in certain grave forms of malarial infection. As to the polycholia, long experience has shown that in pernicious infections the gall bladder is distended with dense and highly colored bile, of which a copious amount is also found in the intestines.

The changes in the liver in pernicious infections were observed by the older physicians. Thus Lancisi says: "Primum in iis, qui ob tertianas perniciosas occiderunt, ingens malorum sedes sub aspectum venit in abdomine, ubi omnia livida et potissimum hepar subfusci, ac bilis cystica atri coloris passim occurrerunt."

Stomach and Intestines.—In pernicious fevers the condition of these organs varies. In comatose perniciosa the only alteration found is an occasional slight melanosis, while in the choleraic form we always find grave changes in the gastroenteric apparatus. In the bodies of patients who have died from choleraic pernicious fever we find in the intestines a fluid which is often sanguineous and contains abundant flakes of mucus. The mucous membrane of the stomach and small intestines is swollen and of a dark red color, sometimes chocolate colored, against which the gray and sometimes swollen Peyer's patches and the solitary follicles stand out prominently, just as do the Malpighian follicles upon the melanotic surface of the spleen.

A microscopical examination of sections of the stomach and intestines demonstrates that the capillaries of the mucosa and especially of the villi are filled with parasites in the various stages of their existence, sometimes in one special stage only, or even in sporulation, mingled with which we find melaniferous and globuliferous leucocytes; these last-named cells may obstruct the capillary lumen. They are found in greater numbers in the veins. The epithelial cells are often necrotic, and even the tissue of the mucosa, especially that of the villi, is the seat of a superficial but extensive necrosis, in the midst of which we can easily recognize the course of the blood-vessels by the pigmentation of their endothelium, and later also that of the parasites which, as a rule, have lost their property of staining. Below the necrotic zone filled with bacteria, we may observe an infiltration of leucocytes. Not infrequently we meet with bodies undergoing karyokinesis in the epithelium at the bottom of the crypts of Lieberkühn—a very evident sign of a regenerative process.

This endovascular condition of the mucosa is in contrast to that of the submucosa and other intestinal coats, whose blood-vessels contain nearly normal red corpuscles and many pigmented leucocytes.

The necrotic changes in the intestine are explained by the slowing

of the circulation in the capillaries of the intestines, which may go so far as to reach corpuscular stasis, and thrombosis of parasites and phagocytes.

Kidneys.—A macroscopical examination of the kidneys in cases of acute infection rarely shows any changes, although we may sometimes find signs of cloudy swelling, or the kidneys may be markedly hyperæmic, or there may be found punctiform hemorrhages of the mucosa of the pelvis and the calyces.

Microscopical examination with a low-power lens often shows pigmentation of the glomeruli and sometimes even in the course of the intertubular capillaries. Under a high power we see that the pigment granules are situated within endovascular leucocytes, and even in the endothelial cells. Rarely we find parasites within the vessels of the glomeruli, but we often find them at all stages within the intertubular capillaries, mixed with melaniferous leucocytes, but in less number than in the organs previously studied. In the renal veins they are few in number or altogether absent. Although few parasites are found in the kidneys, there are in some cases marked changes in the parenchyma. These consist, in the glomeruli, of degeneration and exfoliation of the epithelial cells, and of a cellular and albuminous endocapsular exudation; in the epithelium of the convoluted tubules, of necrosis; in the straight tubules, of hyaline, granular, and epithelial casts. In kidneys thus altered there is no increase in the accumulation of parasites or in pigmentation—a fact which would lead one to infer that the origin of the changes is toxic as it is in other infective diseases.

A chemico-histological examination of the kidneys has been made in several cases with the view of ascertaining whether any reaction of these cells could be obtained with iron. By the potassium-ferrocyanide and hydrochloric methods a reaction was always obtained, which was more marked than in non-malarial kidneys. The reaction was diffuse, but in some instances was more pronounced in the cortical substance, either in the protoplasm or in the nucleus of the epithelium. It is to be noted that the reaction was obtained only after long immersion in the solution of hydrochloric acid. With carbon disulphide there was no reaction in any special portion, but a diffuse greenish-brown coloration was obtained.

In pernicious infections, according to Barker's observations, the same intravascular and endothelial changes are found in the suprarenal glands as in the liver.

In a case of pernicious fever studied by Dock, the *abdominal fat*, especially the peripancreatic, perirenal, and perigastric fat, contained a large number of red corpuscles with parasites within the capillaries

and small veins. In the larger veins were many leucocytes and thrombi of leucocytes in a fibrinous network, the leucocytes containing pigment and parasites either sporulating or ready to sporulate.

In a case of pernicious fever which recently came under our own observation, in addition to many parasites found in the brain, spleen, bone marrow, villi of the intestinal mucosa, etc., the abdominal fat was found to be of a dark reddish-yellow color. A microscopical examination showed that the small blood-vessels of the mesenteric fat were filled with adult parasites ready to sporulate, or already sporulating, as in the organs already studied. As regarded the abundance of parasites, there was no difference between the vessels of the mesenteric fat and those of the brain. It is probable that a systematic examination of the abdominal fat in cases of pernicious fever would show the presence of parasites to be of frequent occurrence, the more so as slowness of the circulation such as is found in the blood-vessels of this tissue is a favorable if not an essential condition to the sporulation of the estivoautumnal parasite.

Bone Marrow.—A macroscopical examination of the marrow of the short and flat bones, as the ribs, for instance, shows it to be of a reddish-brown color like that of the spleen. That of the long bones, as the femur, varies in appearance according to the duration of the infection. In recent infections it is of a normal yellow, in those which have lasted for several weeks the marrow is a brownish-red in the upper and lower thirds of the bone. When the infection is of two or three months' duration, the marrow is of a diffuse reddish-brown or slaty color. The consistence varies. It is sometimes soft and almost diffluent. In chronic cases it is less soft, and therefore can be more easily examined in sections.

The microscopical examination of a fresh preparation shows a number of macrophagi rich in granules, blocks, and lumps of pigment, and red corpuscles containing parasites in the various stages of their existence, from the non-pigmented amœba to the sporulating form. Parasites, even in sporulation, are found to a large extent in a free condition, and there are free lumps of pigment as well. Not only are these parasites in all the febrifacient phases, but also in the crescent stage, especially if the infection has lasted several days. These crescent forms are found in the bone marrow even in cases in which they were not found during life in the blood, or in which but few were found, and in which there was but a small number in the spleen, and none whatever in other organs, as the brain. This fact leads to the conclusion that the development of the crescent stage in the estivoautumnal parasite occurs chiefly, if not exclusively, in bone marrow, this being the tissue most favorable to its growth.

An examination of sections of bone marrow gave in certain cases the following results: The small vessels were filled with endoglobular parasites in the stage which terminates in sporulation, as well as in the crescent stage. In addition they contained globuliferous or pigmented macrophagi clinging to the vascular walls. Between the red corpuscles were seen small ovoid or rounded bodies which from their shape and reaction towards staining-agents were seen to be free spores. Outside of the blood-vessels, among the cells of the marrow, were found a variable number of parasitic forms in the amœboid and in the crescent stages. The globuliferous and pigmented macrophagi were always abundant, and some of them, as in the spleen, were necrosed. There were also medullary cells, for the most part non-pigmented, either undergoing karyokinesis, or with budding or fragmented nuclei. Nucleated red corpuscles were more or less numerous and never contained parasites.

We have now described the conditions found in the majority of cases of pernicious fever in the organs mentioned above. In the other organs (pancreas, genitalia, lymphatic glands), in the muscles, and in the skin few parasites are found, partly because in many of these parts the capillaries are found emptied of blood after death.

We must add to our descriptions of the parasitic condition of the individual organs that the parasites are not always found in equal numbers in all the viscera. On the contrary, the *distribution of the parasites* is not uniform, and their numbers vary greatly in the various organs. In regard to this distribution of parasites which is of the greatest parasitological and clinical importance, we may make the following observations:

1. There are certain cases in which the parasites are found in great abundance in the blood and in all or nearly all the organs. We say nearly all because sometimes the number may be scanty—in the brain, for instance.

2. In some cases there is absolute or relative scarcity of parasites in the bone marrow, spleen, liver, and even in the blood, while the other organs abound in them. The localization is apt to be as follows:

a. The brain and meninges are filled with parasites chiefly or entirely of one life stage, or else in all stages. This cerebral localization is frequent, just as pernicious fevers with cerebral symptoms are frequent. In some cases parasites were found with the greatest difficulty even in the hæmatopoietic organs.

b. The stomach and intestines are usually invaded. This is the gastroenteric localization which manifests itself in the form of pernicious fever with gastroenteric symptoms.

Further investigations will show whether other localizations occur in pernicious infections.

Quartan and Tertian Fevers.

The pathological anatomy of mild malarial infections—the quartan and tertian—can be studied only in cases in which death occurs from some intercurrent disease while the malarial infection is in full force, since the latter does not prove fatal.

The clinical and hæmatological study of these fevers, the presence of a splenic tumor, the anæmia and cachexia (the latter especially in babies and young children) show us that even in mild malarial infections we have the same fundamental conditions in the blood and in the hæmatopoietic organs. But even with due allowance for the lesser toxicity of the quartan and tertian parasites, the clinical data and the examination of the blood lead us to infer that in these fevers there is not the same tendency to unequal accumulations, to visceral localizations of the parasites, that there is so apt to be in pernicious fevers, and which occasions the symptoms peculiar to this type of the disease. The reasons why this accumulation does not occur must be complex. There must first be taken into account the biological property of the parasites which allows them to complete their life cycle in any situation, even circulating freely in the peripheral blood, whence much less injury is inflicted upon the elasticity of the infected corpuscles. Further researches will show whether there are other factors which prevent the unequal distribution of the quartan and tertian parasites. The two varieties behave somewhat differently in this respect, that is to say, the quartan parasites are equally distributed in the various vascular areas of the body at all stages of their existence, while, according to the results of investigations by Bignami, Bastianelli, Antolisei, Barker, and others, the tertian parasites in the fission stage tend to accumulate in the spleen. An examination of splenic juice from patients suffering from tertian fever shows that during the period of invasion the sporulating forms are abundant in the spleen, but scanty in the blood of the finger. Splenic juice examined during the same stage in quartan fever shows the same number of fission forms as is found in blood from the periphery.

Anatomicopathological studies, as we have already remarked, can rarely be made in these mild fevers. In two autopsies made by us in cases of active quartan in which the patients died respectively of nephritis and of spinal disease, the enlarged spleen was found not to be softened nor very melanotic, nor were the liver and bone

marrow markedly melanotic. In one case the parasites were found in the spleen and in the blood, but not in the brain.

Baker's researches are a valuable contribution to the pathological anatomy of this disease. The case which he investigated was that of a youth of twenty-three years, who, three months before his admission to the hospital, had contracted nephritis from having had to stand for two weeks with his feet in cold water while he was at work. Two months and a week later, when the renal symptoms were manifest (bloody urine, œdema, labored breathing, etc.), he began to suffer from quotidian fever which was ushered in by chills. Upon his entrance into the hospital, the urine was found to contain albumin and casts. The fever continued, anasarca supervened, and the patient died seventeen days later. The autopsy showed subacute nephritis, erysipelas, streptococcic septicæmia, tertian malarial infection, and melanosis of the viscera.

In the blood of the heart were found two generations of tertian parasites in *enormous* numbers. A microscopical examination of the *liver* demonstrated within the dilated capillaries phagocytes of various size, some of gigantic porportions, containing pigment, parasites, red corpuscles with and without parasites, and débris of red cells and leucocytes; there were also parasites, either free or within leucocytes. Some of the endothelial cells were so swollen with pigment and parasites as almost to occlude the capillary lumen. Kupfer's cells were also pigmented. The hepatic cells contained rusty pigment. In the spleen there was found hyperæmia of the pulp, with an increase of leucocytes in the trabeculæ of the pulp, in the veins, and in the capillaries. Some among these leucocytes were found to be globuliferous, parasite-infected, or pigmented. Within the blood-vessels were tertian parasites, free, within red corpuscles, or within leucocytes which were for the most part uninuclear, but a few of which were multinuclear. Moreover, in the capillaries, veins, and pulp were macrophagi containing pigment, parasites, débris of parasites and of red corpuscles, and small phagocytes within the macrophagi. The endothelial cells were altered as in the liver. In the *kidneys*, in addition to the lesions of nephritis and the invasion of streptococci, parasite-infected and pigmented leucocytes were found in the intertubular capillaries and in those of the glomeruli. In the *bone marrow* the parasites were nearly all within uninuclear leucocytes. In the *lungs* the veins and the capillaries contained parasite-infected red corpuscles and parasite-infected and pigmented multinuclear leucocytes. In the *suprarenal bodies* the arteries and the veins contained parasites; some veins were filled with uninuclear and multinuclear parasite-infected leucocytes. The capillaries contained macrophagi possess-

ing the same characteristics as those found in the liver and in the spleen, and not equally distributed throughout the capillary network. The endothelial cells were like those of the liver as regards phagocytosis.

From these observations of Barker we see that the changes in the blood and in the hæmatopoietic organs, in mild infections, are in all essentials the same as in estivoautumnal infections. Another important fact is demonstrated, namely, that in spite of the "enormous" number of tertian parasites in the blood of a nephritic patient, and in spite of a streptococcic septicæmia there were no pernicious symptoms, although the infection had gone for three or four weeks without diagnosis.

Chronic Malaria—Malarial Cachexia.

When malarial infection has lasted for a long time in the human organism, either because the germ continues its existence and causes frequent relapses of fever, separated by intervals of varying length, or because the malarial infection, after extinction, is repeated several times, we say that the malaria is chronic. In this case, as in that of cachexia, there are alterations in various parts of the body, which have been the object of investigation both before and since the discovery of the parasite.

A perusal of the not scanty literature upon the subject will convince us that among the malarial lesions properly so-called have been included others which have nothing to do with malaria, such as suppurative inflammations, for instance, and that the pathogenesis of the true malarial lesions has been built up in an arbitrary fashion, instead of resting upon a foundation of minute and exhaustive investigation of the anatomicopathological data. The fact cannot be denied, however, that many malarial lesions have been scientifically studied, especially by Frerichs, Kelsch and Kiener, Forsyth Meigs, Tommasi-Crudeli, Laveran, and others.

Frerichs describes the acute enlargement of the liver and the atrophy following it, considering both to be due to the changes in the blood-vessels caused by the accumulation of pigment. It is to be remarked that the atrophy described by this eminent clinician was found in patients who had died in marasmus after severe diarrhœa. Lancereaux describes cases of malarial cirrhosis in which the liver is not granular and is increased in size. Colin gives the same description.

Laveran describes chronic enlargement of the spleen, and two kinds of chronic lesions in the liver—inflammatory congestion and

cirrhosis which is not to be distinguished from ordinary atrophic cirrhosis. Kelsch and Kiener give a more detailed description of the organic lesions in chronic malaria. First of all, they make a distinction between those found in persons who have had repeated attacks of malaria and those in cachectics properly so called. In the former, the fundamental changes in the liver and spleen consist in a phlogistic hyperæmia due to the abnormal irritation and the functional hyperactivity produced by the incessant discharge of pigment into the organ. In the spleen they describe the melanosis, in which the distribution of pigment does not greatly differ from that in acute malaria, the hyperæmia, the tumefied condition of the pulp, the hyperplasia of the fibrous trabeculæ, and the yellow pigmentation of the cells of the parenchyma. In the liver they describe hyperæmia, and an increase in consistence and size in some cases, and in others a mammillated aspect, induration, and exaggeration of the acinous divisions. Under the microscope they found hypertrophy and hyperplasia of the hepatic cells and yellow pigmentation of the same, especially at the periphery of the acini, dilatation of the capillaries, and changes in the endothelium. In cachexia they distinguish three varieties of cases: 1. Those with an overload of ferruginous pigment in the organs, cases in which there is an enormous splenic enlargement; 2. Cases with atrophied organs (excepting the spleen which is enlarged), especially the liver, with ascites, anasarca, and diarrhœa; 3. Cases with amyloid degeneration. Kelsch and Kiener further describe a true malarial hepatitis, which may end either in resolution or in cirrhosis.

The chief and most characteristic changes to occur in chronic malaria and those of most importance clinically, are found in the spleen, liver, and bone marrow. We will consider these in detail, not omitting those of the other organs which are certainly due to malaria, basing our descriptions chiefly upon the investigations made by Bignami in the Institute of Pathological Anatomy in Rome. For a full comprehension of the pathological anatomy of chronic malarial infection, it is essential that we bear in mind the conditions developed in the blood from the invasion of parasites, and those following in the spleen, bone marrow, and liver from the deposits of the detritus composed of the cadavers of parasites, the remains of red corpuscles, of pigmented globuliferous and parasite-infected phagocytes, desquamated endothelium, etc.

Spleen.—The reader will recall the description of the changes in acute enlargement of this organ, consisting chiefly in hyperæmia, the deposit of a large number of altered red corpuscles, the accumulation of pigmented, globuliferous, and parasite-infected leucocytes, the in-

clusion of parasite-infected red corpuscles in some of the cells of the pulp (by traversing which the blood appears to purify itself, as may be seen by a comparative examination of the parasitic contents of the capillaries and of the veins of the spleen), and the karyokinesis of the pulp cells themselves.

From a knowledge of these conditions in acute enlargement and from the fact that the causative conditions are repeated many times, we are in a position to understand chronic enlargement. The organ may be of excessive size, weighing several kilograms and filling the greater part of the abdominal cavity, displacing and compressing the surrounding parts. The color varies with the persistence of the melanosis, and according to the degree of thickening of the capsule; it may be equally distributed, or more marked in certain parts which have a cartilaginous appearance, or are even calcified. Adhesions are never lacking in chronic enlargement; they are sometimes slight and easily torn, sometimes most tenacious. The cut surface of the organ is smooth, of a slaty or dark or pale red color, upon which, in melanotic cases, the Malpighian follicles stand out with great distinctness. In advanced cases we usually find a pronounced connective-tissue network which is more marked towards the periphery.

The histological changes in the spleen during the various stages of chronic enlargement may, according to Bignami, be summed up as follows: When the acute hyperæmia of the spleen ceases, permanent alterations and reparative processes occur around the accumulations of necrotic elements and the necrosed area of the splenic pulp. The permanent changes consist in the formation of venous lacunæ, separated from each other by thin layers of splenic pulp, and, where the destructive processes have been most serious, by the formation of a tissue composed of enormous cavernous sinuses, separated by an extremely fine connective tissue rich in giant cells, tissues which take the place of the necrosed pulp of the spleen. Some of the Malpighian follicles become necrosed or undergo fibrous degeneration. The processes of repair are seen in the splenic pulp, but to a still greater extent in the Malpighian follicles. The latter become three or four times larger than the normal size, and from them proceed bands of newly formed lymphoid tissue which surround the necrotic zones that gradually disappear. Around the hyperplastic follicles there is hyperplasia of the pulp cells, the reticulum of which becomes thickened.

As to the pigment, this, probably with many necrotic cells, is carried away by the lymphatics, and at first accumulates towards the periphery of the follicles, for which reason diffuse melanosis is fol-

lowed by perifollicular melanosis. The pigment then travels off by means of the lymphatics of the arterial coats and those of the connective-tissue system of the septa, the pigmentation of the splenic tissue thus gradually diminishing until it finally disappears. As a result of this migration of pigment there is thickening of the coats of the vessels and splenic septa on the one hand, and on the other the formation of lymphatic cysts which may be isolated or multiple and accumulated in such a way as to resemble true lymphangiomata.

The formation of the sometimes enormous splenic enlargements, which are so often observed in patients who have long suffered from malarial infection, will readily be understood if we bear in mind that the above-described modifications occur with every new acute infection, that is to say, there are new necrosis, new formation of angiomatous tissue and of follicular tissue, new deposits of pigment, and migration of the latter through the lymphatics, whence arises the progressive thickening of the perivascular coats, the connective-tissue septa, and the capsule of the spleen.

Liver.—As in the case of the spleen so in that of the liver, to arrive at a full comprehension of chronic enlargement and its pathogenesis it is necessary to start with the acute swelling, in which we find an accumulation of phagocytes in the capillaries, phagocytosis of the endothelium with retrogressive changes and exfoliation, retrogressive changes in the hepatic epithelium (nuclear changes, necrosis of the cellular bodies, yellow pigmentation of the same), etc. Now when these conditions are frequently repeated for months and years, chronic alterations of the liver occur. These alterations will be more perfectly understood by classifying them according to the presence or absence of melanosis, and in the melanotic liver according to the distribution of the pigment—in other words, by following their development.

In a melanotic liver we distinguish the following stages: 1. The liver becomes congested, the lobules are not distinctly seen, and there is more or less marked diffuse melanosis. Under the microscope we find dilatation of the capillaries, the endothelium is for the most part pigmented, especially at the periphery of the lobules, many of the endothelial cells show chromatic changes in the nuclei, and some are necrotic. Within the capillaries may still be seen a few pigmented or necrotic macrophagi. The perivascular lymph spaces are enlarged. Outside of the capillaries are seen pigmented Kupfer's cells, uninuclear and multinuclear leucocytes, and pigmented cells with a large nucleus. In the connective tissue of the triangular spaces are accumulations of endocellular or apparently free pigment around the arterial and venous blood-vessels and the bile ducts.

In the hepatic cells are seen retrogressive and progressive changes —the former shown by a deficiency in the coloring of the nucleus, the swelling of the protoplasm and necrosis of the nucleus, the overloading of the protoplasm by lumps of yellow pigment, fatty degeneration, atrophy, and deformation of the cells. The progressive changes consist in the presence among the hepatic epithelium of bodies undergoing karyokinesis. It is to be noted that among the altered cells some normal ones are found.

2. In a more advanced stage the melanosis is less diffuse, and predominates towards the periphery of the lobules. A microscopical examination shows extensive dilatation of the capillaries; enormous dilatation of the perivascular lymph spaces which contain leucocytes with polymorphous nuclei, and grains and lumps of pigment; marked pigmentation of the perilobular connective tissue where many nuclei are seen around large blocks of pigment; zones of necrosis of the hepatic cells, and spots where these cells have entirely disappeared and are replaced by a supporting connective-tissue reticulum and pigmented Kupfer cells. The most important condition in this stage is the beginning of the new formation of hepatic cells. This process usually begins towards the middle of a lobule, whence it extends throughout a large part or even the whole of the lobule. The newly formed hepatic cells are arranged as cellular cords by the side of the remnants of the necrosed hepatic cells, around which are accumulated multinuclear lymphocytes, some containing pigment, others clinging to the necrotic detritus in such a fashion as to suggest the idea of a phagocytic function destined to carry away this detritus which would then be replaced by the young hepatic cells. In the parts where the regenerative process is evident, the capillaries have normal endothelium and there is no pigmentation. In necrotic zones involving the stroma, there are no signs of regeneration, as in those in which the stroma and the capillary network of the lobes are preserved.

In the parts which are the seat of regenerative processes, we find giant cells with budding nuclei, like those in the bone marrow, intravascular or clinging to the vessel walls. They are like those found in the embryonal liver (Foà and Salvioli), spleen, and bone marrow when the hæmatopoietic functions of these organs are most active.

3. The diffuse melanosis is followed by an exclusively *perilobular* melanosis. In this stage the liver is enlarged, its consistence is somewhat increased, and its surface is smooth. Upon this surface as well as on the cut surface we see that all the lobules are distinctly outlined by thin slate-colored lines, so that it is easy to see that they vary greatly in size, some of them being two or three times as large as normal.

Under the microscope the most important conditions found are atrophy of some lobules with compensating hyperplasia of others; pigmentation most pronounced towards the periphery in the form of large black and rusty masses contained in endothelial cells whose nuclei have become altered, in Kupfer's cells, or in other perivascular cells, showing a tendency therefore to leave the blood-vessels; the neoformation of endothelial cells which so arrange themselves upon the necrotic and pigmented cells as to make these extravascular. Around the lobules, and especially in the triangular spaces are seen accumulations of cells containing globules of pigment decolorized at the centre, which are also found free. Permanent lesions of two kinds have been found in extensive zones of lobules, namely, the formation of lacunæ and lymphatic cysts lined with endothelium, which, when in groups form true lymphangiomata; and false angiomata formed by the conversion of the capillary system into a system of large vascular lacunæ containing many white cells, in which, when the lesion is at an advanced stage, no traces of hepatic cells can be found.

4. When the infection has been spent for several months, the melanosis is so greatly diminished as to be scarcely visible to the naked eye. The liver is enlarged and congested, the lobules are very distinct, surrounded by a slender brownish ring, and the consistence of the organ is somewhat increased.

A microscopical examination shows that the melanosis is entirely perivascular, consisting in endocellular blocks of pigment, especially around the perilobular vessels of small and medium calibre, and in smaller amount around the vessels which traverse the triangular spaces. We notice, moreover, that there is hyperplasia of the perivascular connective tissue, of small extent in the triangular spaces. The hepatic lobules vary in size. The alterations in the parenchyma have, in large measure, been repaired. The capillaries remain somewhat enlarged. In some lobules we find lymphatic ectasia, and false blood angiomata.

When the pigmentation has disappeared, many of the above-described alterations remain, constituting the chronic malarial hepatic enlargement which is so often found accompanied by enlargement of the spleen in persons who have suffered for months or years from malaria, and which may persist for months and years after the infection is gone. The liver is increased in size and may weigh as much as four or five kilograms. The surface is smooth, the capsule is thickened. A cut surface shows distinctly and prominently lobules surrounded by a ring of pinkish tissue.

A microscopical examination shows the absence of melanosis, hyperplasia of the perilobular connective tissue, dilatation of the capil-

laries with stasis of leucocytes, hyperplasia of some of the lobules, in some cases persistence of the lymphatic cysts, and false angiomata.

The pathogenesis of the lesions causing the chronic enlargement is easily inferred from the description given. The hyperæmia accompanied by stasis of leucocytes by which the process begins is explained by the changes in the endothelial cells. The circulatory changes account for the retrogressive changes and the necrosis of the epithelial cells of the glands, which are in part repaired by regeneration of new epithelial cells, and in part followed by angiomatous and lymphatic dilatation. The latter is probably chiefly due to occlusion of the perivascular lymphatics by large melaniferous phagocytes. In addition to the circulatory changes it is possible that the yellow pigmentation of the hepatic cells, which is sometimes very marked, may contribute to their atrophy.

Authors are divided in their opinion as to the time necessary for the disappearance of the pigment and the method of its elimination. From Bignami's researches it would appear that three to four months are required. The pigment is carried from the blood-vessels into the lymphatics by means of large uninuclear leucocytes, and even by multinuclear leucocytes or those with polymorphous nuclei which take the pigment from the necrosed endothelium and macrophagi. While they are carrying it, it is in part transformed and destroyed by endocellular digestion.

The hyperplasia of the perilobular connective tissue is caused by the continual deposition along the lymphatics of the detritus of parasites, and of the many disintegrated cells, and of black pigment, which, perhaps because it is the most conspicuous element, has been held to be the principal factor in the hyperplasia.

The regeneration of the hepatic cells has been described. The great regenerative power of the hepatic parenchyma has been clearly demonstrated by recent experiments, especially by those of Ponfick; a regeneration which, according to all authorities, occurs from hyperplasia and hyperæmia of the hepatic cells, without the formation of new hepatic lobules.

From the description which we have given of malarial hepatomegaly, we can easily judge of the difference between it and hypertrophic cirrhosis, the only form of cirrhosis capable of causing any perplexity as to differential diagnosis. In this affection the liver is increased in size and consistency and is always markedly icteric. The connective-tissue hyperplasia is annular, with abundant new formation of bile ducts, while the hepatic cells are icteric but intact. In malarial hepatitis there is no icterus, perilobular connective-tissue neoformation is scanty as is that of the bile vessels; the capillaries

are dilated, and there is stasis of leucocytes from slowness of the circulation. The hepatic cells show many and various alterations.

From a study of all those conditions which are met with in a chronic malarial hypertrophy of the liver, as well as from the symptoms, Bignami has come to the conclusion that chronic malarial enlargement of the liver is not a cirrhosis nor a chronic hepatitis in the proper meaning of the word, but a process which differs from that of any cirrhotic processes known, anatomically, pathogenically, and clinically.

A much disputed question is whether malarial infection can cause ordinary cirrhosis. Even the writers who have pursued their studies in malarial regions differ on this point. Frerichs holds that when there is cirrhosis, some other cause must be invoked, in addition to the malaria. Colin and Laveran believe that cirrhosis is very rare in malarial patients. Kelsch and Kiener give a long description of malarial hepatitis, and distinguish three forms: 1. Nodular hepatitis with hyperæmia, characterized by the formation of nodules of proliferating epithelium which may terminate in central necrosis or in purulent softening (?); 2. Nodular hepatitis with cirrhosis; 3. Nodular hepatitis with adenomata.

To these three forms of chronic hepatitis they add two others which do not differ in appearance from ordinary cirrhosis. It is unnecessary to enter into a critical review of this portion of Kelsch and Kiener's work; we would merely state that their classification of malarial hepatitis is made according to the schematic method of the French authors in their study of cirrhosis in general, and that many of the cases from which their descriptions were taken were in malarial patients who at the same time were alcoholic or dysenteric. In Italy such eminent clinicians as Tommasi, Cantani, and Cardarelli have upheld the importance of malaria in the production of hepatic cirrhosis.

Basing our opinion on the observations made in the Roman hospitals, we, however, believe that malaria does not produce cirrhosis in general, and ordinary cirrhosis in particular. Nor do we believe that the chronic hepatic enlargement of malaria can be considered as the first stage in the cirrhosis of Laennec, because we have to do with two entirely different histological processes. Osler is of the same opinion.

If malaria were alone the cause of hepatic cirrhosis it should be found with greater frequency in malarial regions and in patients who have for a long time suffered from the infection. Now this is not the case, as we are able to assert from an experience covering many years; and in the several cases of ordinary cirrhosis which we are

called upon to examine every year, we do not find in the enlarged spleen any of the characteristics of malarial infection. Moreover, the anatomicopathological lesions are quite different; in fact, due consideration of the initial lesions of the malarial enlargement will enable us to recognize the fact that its ulterior development cannot lead to ordinary cirrhosis.

By all this we do not wish to deny that atrophic cirrhosis and hypertropic cirrhosis with icterus may develop in patients with chronic malaria, but we do mean that other causes are necessary to their production. Finally, it is to be remembered that malarial infection may develop in cirrhotic patients, and that therefore we may have a cirrhotic liver with melanosis and the other lesions described above, without being obliged to hold that the cirrhosis is of malarial origin.

But, if there are no reasons for believing that there is an ordinary cirrhosis due to malaria, we must admit that there are grave atrophies of the liver, which are directly or indirectly derived from the malarial infection. Bignami, who has studied them recently, describes two varieties: *simple atrophy of the liver in malaria, secondary to thrombosis of the portal vein*, and *simple or marantic atrophy of the liver in malaria*. These atrophies are usually found in old people suffering from malaria who have died from exhausting diseases, or in malarial patients with progressive anæmia, etc. In these cases the liver is notably smaller, the surface is usually finely granular or smooth, the capsule is thickened, the consistency is increased; the lobules are small and distinct. A microscopical examination shows grave retrogressive changes of the hepatic parenchyma, scanty neoformation of connective tissue, no signs of a regenerative process. Thrombosis of the portal vein is found in old people who are malarial, anæmic, suffering from diarrhœa, etc. Ascites is apt to form rapidly and in great abundance.

One special retrogressive change of the liver, whose dependence upon malaria is in many cases very evident, is *amyloid degeneration*, in chronic hypertrophy as well as in atrophy. This degeneration is also found in other organs, as we shall see presently, the patients presenting the signs of cachexia with dropsy, diarrhœa, etc.

When treating of the pathogenesis of chronic enlargement of the spleen and liver, we spoke in detail of melanosis, and called attention to the ochraceous pigmentation, the importance of which was pointed out by Kelsch and Kiener. As to the genesis of the melanæmia there is no longer any doubt; the black pigment is formed within the body of the parasites from the hæmoglobin of the invaded red corpuscles, and this black pigment is as we know deposited in the hæmatopoietic

organs. The yellow pigment, which is found in large amount within the splenic and hepatic cells, or in a free state, is derived partly, if not wholly, from the red corpuscles previouly altered by parasitic invasion (brassy bodies). This pigment gives the well-known azure reaction when sections of the organ are treated with potassium ferrocyanide and hydrochloric acid. This reaction, which is extensive in acute malarial enlargement of the spleen and liver, is less so in the chronic condition. From this fact and from others, it seems very probable that the yellow pigment is transformed in part into black pigment. In the liver the pigment is certainly used in part in the formation of bile.

Bone Marrow.—The changes in the bone marrow may be divided into two kinds, namely, those secondary to acute infections and those secondary to the anæmic condition produced by the infection.

In patients who have had many relapses of malarial fever, the marrow of the long bones, as the femur, is usually red, and in the upper and lower thirds the consistency is greater than in acute infections, while the middle third has the yellow appearance of adult marrow, and only gradually becomes converted into red marrow. A microscopical examination shows disappearance of the adipose tissue, which is replaced by a rich and very vascular medullary tissue. In this tissue we find large cells with abundant protoplasm and vesicular nucleus, even in mitosis; cells like the preceding but with a uniformly stained nucleus; lymphoid cells sometimes gathered in groups around the blood-vessels; giant cells with gigantic and budding nuclei in great number; nucleated red cells usually of normal size (normoblasts). There is a rich capillary network; the walls of both arteries and veins are thickened. The pigment disappears rapidly from the bone marrow, and in fact is found there in only scanty amount when the melanosis of the spleen and liver is still intense. These changes in the yellow marrow, like those which occur in anæmia from blood-letting, etc., show that there is an awakening in the hæmatopoietic activity of the bone marrow.

The minute details of the transformation of the yellow marrow into active marrow have been revealed by the recent investigations by Bignami of the transitional zone between fatty and red marrow in the diaphyses of the long bones. In these zones we observe two chief facts, namely, the development of a rich network of vessels, partly capillary, partly forming true lacunæ between the fat cells, and a progressive shrinking of the fat cells. While the latter is taking place, there appears at the periphery of the fat cells a zone of an amorphous or finely granular substance, which, arranging itself in concentric layers, gradually fills up the space occupied by the cells, and in its

turn becomes gradually thinner and finally disappears, to be replaced by the round cells with polymorphous nuclei, and then by the red corpuscles which take up all the space previously occupied by the cells. Thus we have the formation of new capillaries and of lacunæ lined with endothelium—lacunæ which at a later stage become filled with the various kinds of medullary cells. The stages passed through in this process are then the following: Fatty marrow, telangiectatic marrow, hæmatoblastic marrow. It is to be noted that when the malarial parasites are present in large numbers in the blood and the organs, they and the phagocytes are to be found in the small veins and capillaries, but not in the lacunæ, in which are the normal red corpuscles and leucocytes. This fact suggested the idea to Bignami that the blood of the capillaries and veins passes into the lacunæ purified from the parasites and phagocytes by some mechanism as yet unknown.

There are cases in which the infection is so frequently repeated as to lead to a cachectic condition, in which the awakening of the functional activity is insufficient to compensate for the continual loss of red blood corpuscles. The marrow then presents the appearance already described. In the long bones all of the marrow is red, but nucleated red corpuscles may be scarce. There are also some rare cases in which a progressive pernicious anæmia follows the malarial infection, from a pathogenesis as yet not well defined. In these cases, the bone marrow varies in appearance. Sometimes the microscopical characteristics are analogous to those found in pernicious anæmia, that is to say, there is notable hyperplasia of the medullary cells, and large numbers of giant red cells (*megaloblasts* or *gigantoblasts*, Ehrlich) are present. In other cases the marrow remains chiefly fatty; in other words, there is no reawakening of functional activity, whence the progressive anæmia.

In malarial cachexia with amyloid degeneration an examination of the bone marrow gives results similar to those in the latest class mentioned above, in addition to which there is amyloid degeneration of the medullary arterioles.

Amyloid Degeneration.—We have now described the most characteristic changes which are to be met with in chronic malarial infection and cachexia, but it is scarcely necessary to add that we have not deemed it essential to describe such changes as are constant or frequent in all chronic anæmias and cachexias, nor such as are merely complications, although some writers consider them to be directly related to malaria. We would, however, say a word or two in regard to a diffuse retrogressive alteration, which, although not absolutely characteristic of malaria, often results from it, namely, amyloid

degeneration. It is not a rare thing to find amyloid degeneration in the organs of those who have never been subjected to any other infection than the malarial. In the clinical history of these cases we find that a long series of estivoautumnal fevers is followed by a cachexia of rapid course with nephritic symptoms, the patients dying in the course of a few months. At the autopsy the chief conditions noticed are grave anæmia, a marantic state of the organs, chronic nephritis, and diffuse amyloid degeneration.

The diffusion and distribution of the degeneration, in the cases so far studied, are as follows: 1. It is most marked in the kidneys, where it attacks not only the vessels of small and medium size, and the glomeruli, but to a great extent also the walls of the renal tubules. The alterations in the interstitial tissue and in the epithelial cells are very great. 2. The intestines and spleen are second to the kidneys in the degeneration. In the intestines the vessels of the villi are chiefly affected, but the vessels of the submucosa and to a less degree those of the other intestinal coats are involved. In the stomach we have the same process; simple ulcers sometimes occur as a result of the degeneration in this organ. In the spleen, the part chiefly affected is the vascular network at the periphery of the follicles, where there are large deposits of amyloid matter, which are absent or in small amount in the trabeculæ of the pulp. 3. In the amyloid degeneration of malaria the liver is apt to be less affected than in amyloid degeneration from other causes. The starchy matter is found in irregularly disseminated patches; for instance, we may find a patch as large as or larger than a lobule in which the hepatic tissue has entirely disappeared and in which the vascular network contains a large deposit of starch, and be surrounded by hepatic tissue of normal appearance. The degeneration seems to begin at the periphery of the lobules.

From the observations so far made, it would seem to be permissible to infer that the amyloid degeneration following malaria, while characterized by a malarial cachexia of rapid course, accompanied by symptoms of nephritis, is anatomically distinguished by the gravity with which it attacks the kidneys, and secondly the intestine and spleen, while the liver is less extensively affected.

The pathological anatomy of the sequelæ and complications of malarial infection will be considered in the sections treating of these subjects.

SYMPTOMATOLOGY.

Classification of Malarial Fevers.

A scientific classification of the malarial fevers must naturally be based upon the knowledge of their etiology acquired in recent times. We have shown how laboratory researches enable us to distinguish various species of malarial parasites. In the same way a clinical and epidemiological study will enable us to distinguish various forms of malarial fever. We are in a position to state that the results of clinical and epidemiological researches are in perfect accord with the parasitological investigations, and we may consider it as an established scientific fact that the different malarial fevers distinguishable in their typical clinical forms are produced by various species of parasites.

In nearly all the classifications given by recent authors, the quartan, tertian, and estivoautumnal fevers are considered to be produced by different parasites. When divergencies in classification exist, they occur in the manner in which these various species are grouped or contrasted to each other, or in the method of subdivision of the estivoautumnal fevers, or in the name given to the third group (irregular fevers, fevers due to the falciform hæmatozoa, to the hæmamœba præcox, the Laverania malariæ, etc.).

Almost all writers divide the fevers into two groups: one formed of the quartan and tertian types, and the other of the estivoautumnal. Some authors (among whom we include ourselves) view the subject clinically, and consider the first two types as *mild fevers*, and the second group as composed of *severe fevers*. This distinction is of practical importance, as nearly all the pernicious fevers belong to the second group. Since the discovery of the parasites of malaria, nearly every case of pernicious fever admitted to the hospital of Santo Spirito in Rome has been studied bacteriologically, and the estivoautumnal parasite has always been found. The relationship between the clinical forms of pernicious fever and a special parasite—which was evident from the first descriptions of the disease given by Marchiafava and Celli, and absolutely demonstrated by the anatomicopathological researches of Bignami—may now, after numerous confirmations from many investigators, be regarded as an established fact. It is true that many of the fevers of this second group are not grave in their form and may be long protracted without menacing the lives of the patients; it is also true that many cases are seen to improve and recover spontaneously; but this does not diminish the clinical and practical value of the above-given classification. There is no parasite of pernicious malaria, or, rather, no parasite which causes perni-

cious malaria only, as some (for instance, Grassi and Feletti) have held; but the only parasite which can cause pernicious fever is that of the so-called estivoautumnal fever. This fact is of such great clinical value that we may venture to utilize it to characterize the whole febrile group.

We have made this division into two groups, starting from an epidemiological conception, and naming them from the season in which the febrile types composing them predominate. Thus the quartan and the tertian and the more complex fevers to which these types lead, have been united into the group of *winter-spring fevers*, to which we may oppose the group of *summer-autumn*, or *estivoautumnal fevers*. The first name appears to many to be unjustifiable. In fact, the quartan and tertian may be found at all seasons of the year in some malarial countries, and in countries mildly malarial they predominate in the summer and autumn. But it must be remembered that we have adopted this classification from a study of the fevers of the Roman Campagna. Now in this malarial region the quartan predominates in the autumn and winter, the tertian in the spring, when primary infections belonging to the second group (estivoautumnal) are almost or altogether lacking; and in the summer and the autumn, the tertian and the quartan hold a position subordinate to the malarial endemic peculiar to this season. In order to judge of the value of this distinction, it is necessary to view it from the standpoint of a physician, who, in a malarial region like our own, follows the development and the succession of the various forms of malaria. One of the most striking phenomena is the profound change which occurs in the clinical type of the fevers, and the parasitic distribution between the end of spring and the beginning of summer. While in the months of April, May, and the greater part of June the ordinary tertian is in the ascendant, towards the end of June and the early days of July we begin to find cases of a tertian fever clinically entirely different from the first, and produced by a special parasite—the *estivoautumnal tertian*. This new form of malaria predominates in the summer and autumn over all the other varieties. In November, as a rule, except in years when malaria is most intense, primary cases of malaria begin to diminish, while relapses of the same type are frequent. Gradually the estivoautumnal type yields its place to the other varieties of malaria, more especially the quartan. It is only very exceptionally that in the winter we have a primary infection due to the estivoautumnal parasite; we have a report of one example, in which the course was that of a pernicious infection. These facts, observed in regular succession for many years, show so close a relation between the season of the year and the appearance of the various forms of fever that it

seems justifiable to take them as a basis of classification. It must, however, not be forgotten, and indeed follows logically from what has been said, that the terms "winter-spring" and "summer-autumn," or "estivoautumnal," are not absolute in their seasonal significance.

This relationship did not escape the acumen of the older physicians. Sydenham divided intermittent fevers into *winter* and *autumnal*, and considered this division so necessary that without taking it into account it would not be possible to give a positive prognosis or to institute proper treatment. Sydenham considered winter fevers to be those which occurred from February to August, and autumnal such as occurred from August to February, and described these two groups as differing from each other in their symptoms, results, and duration; the first he considered as usually, though not always, milder and shorter, the second as graver and more obstinate. He also noted that the winter fevers were not dangerous, while the epidemic autumnal tertian was not devoid of danger and was very resistant to treatment.

We must, however, recognize the fact that this basis of classification has a merely local importance; indeed it could not well be otherwise. It is of value for all gravely malarial countries of temperate climate, in which all forms of the fever are found, alternating and prevailing successively in the various seasons; the Roman Campagna may be considered as an example of such malarial regions. But a classification which is accurate for this region can very evidently not be adapted to countries where the malaria is of a mild type, as for instance Lombardy, nor to tropical malarial countries. In the first-named regions the tertian form, which we have called the spring type, prevails as a rule, while in the second we have the estivoautumnal fevers. The distinction may therefore be maintained only in the group of countries to which we consider it adapted, and cannot be generalized.

Other writers have divided the fevers into two groups, basing their classification upon a study of the biology of the parasites. This is the method of Golgi and Mannaberg.

Golgi distinguishes (1) a group of fevers whose pathogenesis is due to parasites which exist and complete the various phases of their existence chiefly in the circulating blood, and (2) a group of fevers due to parasites situated and developing under relatively stable conditions in the internal organs (especially the marrow of the bones and the spleen). To the first group belong the quartan and the tertian, to the second the fevers which we have denominated estivo-autumnal.

We have elsewhere fully given the reasons why we are unable to

accept this mode of regarding the biology of the parasites of estival fever adopted by Golgi. We cannot admit that the estival parasites complete the whole cycle of their existence under stable conditions in the internal viscera; there is only one phase of their life—their multiplication—which is completed in the viscera, and that not exclusively, but the other phases occur in the circulating blood, as is the case with all the malarial parasites. Without stopping to enlarge upon this point of view, for a study of which we refer the reader to another of our works, we will simply add that if we wished to adopt the above biological basis of classification, it would, in our opinion, be necessary to modify it, and to distinguish the malarial fevers as follows: (1) Fevers in which the parasites *multiply* in the *circulating blood* (tertian and quartan), and (2) fevers in which the parasites *multiply* under conditions of relative stability in the *internal viscera* (estivoautumnal fever).

It is not to be denied that this difference in the seat of development is of great biological importance, a fact which did not escape those who first made researches into the matter. In truth, we may note that the parasites of the ordinary tertian show the same tendency as the adult form to accumulate in the viscera, so that from this point of view they may be considered as a transition stage between the parasites of the quartan and those of estival fever. But even admitting this, from our point of view the differences between the two varieties of fever remain so great that they cannot escape the observation of any one. In the ordinary tertian it is the rule to find sporules in the circulating blood, while in the estival tertian it is the exception. The objection, therefore, is of small import.

In spite of this, it does not seem justifiable in our eyes to raise the above-mentioned fact to the dignity of a fundamental basis for classification. If the parasites of summer fever had a more or less stable seat in some determined viscus, as for instance the spleen, and in this organ alone, this fact would be of fundamental importance, and would permit us to divide the malarial parasites into those situated in the circulating blood, and those, let us say, in the spleen. But such, as we know, is not the case. The parasites of summer fever multiply principally, it is true, in certain organs, but the place in which their multiplication occurs, as shown by a study of the pernicious form, is very variable (brain, intestines, spleen, etc.). We must then believe that this is due to a number of causative factors, among which enter largely the alterations produced by the parasites in the red blood corpuscles and in the endothelial layer of the blood-vessel walls, perhaps also to individual conditions, and not alone to a fundamental biological property of the parasite which must have

a determined seat in which alone it can multiply. Only in case this were true (which it is not) could we believe that this characteristic of the parasite would endow it with sufficient importance to form the basis of a classification. Neither can we adopt the classification of Mannaberg, which is also founded upon parasitic theories. This writer, starting with the idea already mentioned in the first part of this work (page 46) that the crescent bodies are syzygia formed by a fusion of the smaller parasites, divides parasites and hence malarial fevers into two groups: (1) Fevers due to parasites which multiply by scission, and without the formation of syzygia (that is to say, without crescentic formation); this group contains (*a*) the quartan, and (*b*) the tertian. (2) Fevers due to parasites with sporules and with syzygia (that is to say, with crescent bodies). These last correspond to our group of estivoautumnal fevers.

We have elsewhere given the objections which can be urged against Mannaberg's theory concerning the biological significance of the crescent bodies and their genesis. So far as we know, the idea that these bodies are derived from the fusion of several plasmodia has not been positively confirmed by other observers, so that the chief reason for Mannaberg's classification is based upon that which is open to reasonable doubt. We have observed elsewhere that not a few points in the biology of malarial parasites are still *sub judice*. One of the questions most open to discussion is that relating to the significance of the crescent forms and the bodies derived from them, the so-called flagellated bodies. In our opinion, it is best to avoid taking a mere hypothesis concerning the biology of these organisms as a basis for the classification of the parasites and of the fevers produced by them.

From the various attempts at classification above noted, it is easy to see that underlying the differences in basis and in nomenclature there is fundamental harmony; in effect, whether we start from a clinical, epidemiological, or parasitical standpoint, one result remains always the same: namely, the distinction and the comparison between the quartan and tertian fevers, and the group of estivoautumnal fevers. We may consequently conclude that these fevers form two natural groups of fixed individuality and distinct limitations. We may still discuss the theories of their division, but we must accept the result which contains that portion of the truth which is to be found in each of the classifications. We have already mentioned the fact that writers also differ in their mode of subdividing the group of estivoautumnal fevers.

We ourselves distinguish two fundamental clinical types (the quotidian and the tertian estivoautumnal or malignant), to which

belong the continuous or subcontinuous or the irregular fevers, which are so numerous in this category. Mannaberg, while accepting this subdivision which we have made, distinguishes one quotidian type due to a parasite which does not become pigmented from another quotidian whose parasite does take on pigment. The existence of malarial fevers caused by parasites which do not produce melanæmia is admitted by this authority, as it is by Grassi and Feletti, who base their opinions upon the researches of Marchiafava and Celli. In some cases of pernicious fever, these authors have described plasmodia which develop to maturity and multiply, without producing pigment. But that these parasites constitute a species or variety apart in the group of estivoautumnal parasites is a thing which cannot as yet be considered as demonstrated. In fact, in these very cases reported by Marchiafava and Celli, in which all the parasites in the various stages of development, without a trace of pigment, were found in some of the viscera, as for instance the brain, in other viscera there were also found some fission parasites with pigment. The hypothesis that in this case we have to do with a double infection due to two species or varieties, "pigmented and non-pigmented parasites," seems to us to have no foundation, because we know of no well-investigated case of malarial infection in man in which the plasmodia have completed the whole cycle of their existence without becoming pigmented, thus furnishing us with a sample of pure culture of this presumed species of parasite. The cases of pernicious malaria in which have been found a large number of plasmodia in scission without pigment are altogether exceptional, and in the last few years we have not seen a single case. We know that there are some species of parasites similar to those of malaria which do not become pigmented, and which are pathogenic for certain animals (oxen, some birds, etc.), and accordingly we should have no difficulty in admitting the existence of a species of similar parasites which may exceptionally be pathogenic in man; but up to the present time a convincing demonstration is still lacking.

A far more notable and greater difference in the consideration of the estivoautumnal fevers exists between ourselves and Grassi and Feletti. We have already spoken of the classification of the parasites. We would here simply call to mind the fact that according to these writers the fevers which we term estivoautumnal should be divided into two distinct groups; one group would contain the pernicious fevers, produced by parasites with early formed sporules (hæmamœba præcox), or which multiply without pigmentation (hæmamœba immaculata); another group would contain the quotidian, subcontinuous, and long-interval fevers, produced by para-

sites whose most characteristic form is the crescentic (Laverania malariæ).

In other words, all these fevers which we consider as constituting fundamentally only one species of malaria, according to Grassi and Feletti, are not only produced by different species, but by parasitic species belonging to different genera: to the genus hæmamœba would belong the species producing pernicious fever, to the Laverania the others. This decided divergence in opinion is due to different ways of regarding the genesis and the biological significance of the crescentic varieties; but this we have already discussed, giving the reasons why we could not indorse the views of Grassi and Feletti. In relation to the classification of fevers, we would simply add that, apart from the parasitic question, all the clinical observations made by us and by others, following in the path indicated by us in our work upon the subject, completely disprove the possibility of separating pernicious fevers from non-pernicious fevers belonging to the estivo-autumnal group. Daily experience teaches us, on the contrary, that in all malarial fevers—whether pernicious or not—produced by the small estivoautumnal plasmodium, the crescentic form constantly follows the first. This is the natural sequence, as we have shown in the special chapter on the subject. We would further note that other observers do not seem to have followed in the footsteps of Grassi and Feletti in this particular theory.

We may appropriately devote a few words to a discussion of the *long-interval* and the *irregular fevers*, although from clinical and parasitic researches, we cannot admit that these fevers constitute a definite species, and we have consequently not given them a special place in our classification.

As to the long-interval fevers, the reasons why they do not seem to us to constitute a natural group have been stated in a short article by Bignami, whose conclusions we present. We must in the first place distinguish between fevers with long regular intervals and fevers with long irregular intervals. It is to the first variety that the ancient writers, from Hippocrates and Galen to Borsieri especially refer, when they speak of fevers recurring every six, seven, eight, or more days. We can express no opinion in regard to this variety of fever because, since the discovery of the malarial parasites, no one has had the opportunity to observe a single example. The majority of modern clinicians and pathologists either do not mention this variety, or speak of it only to doubt its existence. On the other hand, it is almost impossible at the present time to make a critically accurate examination of the cases recorded in ancient literature. It is known that Galen and Tulpio stated that they had observed a case of quin-

tan fever with a periodically regular course. A sextan is described by Zeviani, who followed the case for a whole winter, thus assuring himself of the regularity of the attacks. After Hippocrates, several of the older writers, as Boerhaave, Werlhoff, Tissot, and others, speak of a septan; Morgagni also mentions it, disputing the opinion held by some that this case must have been a quartan fever the intervals of which had gradually become prolonged. We also find mention of an eight-day, nine-day, and ten-day fever, and finally of a fourteen-day and a fifteen-day variety.

Further accounts of these various discussions may be found in Borsieri, whose work upon practical medicine is rich in quotations on the subject. But although recent data upon these types of fever are lacking, all physicians living in malarial regions are aware of the existence of fevers which recur at long interval (longer than those in the quartan) and are irregular in type. To this category belong a few cases studied by us, and one by Golgi; in the latter there were groups of febrile attacks separated by intervals of apyrexia lasting from five to ten days. As a rule in such cases, we observe two, three, or four daily attacks which seem to be united in groups, followed by intervals of apyrexia varying in the different cases from five, ten, or fifteen days up to a month. Now a microscopic examination of the blood shows that these fevers do not from an etiological standpoint form a group apart; in fact, in some of them we find the estivo-autumnal parasites, that is to say, the small plasmodia during the febrile attack, and often, although not constantly, the crescentic varieties during the apyretic intervals; in others we find the parasites of the tertian or even of the quartan. The majority of physicians consider these fevers to be due to a series of relapses. We also hold that the long intervals of apyrexia which separate the single attacks or the groups of attacks cannot be called intermissions in the true sense of the word, but rather are periods in which the malarial infection is latent. The reasons which lead us to adopt this view will be dwelt upon when we come to consider the subject of relapses in the section on chronic malaria. And indeed, if these fevers are held to be simply a succession of relapses, they represent merely a clinical form of chronic malarial infection, whatever the species of parasite producing them.

As to *irregular fevers*, we cannot indorse the opinion of those writers who consider them to be the same as estivoautumnal fevers. All our researches lead us to affirm the existence in this group of fevers of some regular clinical types, and we find that many investigators are following our lead. The irregularity in these fevers is merely apparent, superficial so to speak, and hides a regularity

which is, however, difficult to recognize. Contradicting the statement that all the estivoautumnal fevers can be called irregular, we may further state that no special type of fever exists which possesses irregularity as its essential characteristic, but that fevers of every class may become irregular in different ways; some febrile types are less, and others, such as the estivoautumnal, more likely to become so. If we recall to our minds the law, that "a regular intermittent fever is produced by the development of one generation or colony of parasites, which are nearly all at the same stage of development," we may even *a priori* form an idea of the various methods in which a febrile type may become complex and irregular. It is also easy to understand that mixed infections, or those given by several species of parasites, may frequently be accompanied by fever of an irregular type. But there is no existing variety or species of parasite characterized by uncertainty and irregularity of development, and hence the formation of a group of irregular fevers has no foundation in the study of parasitic life, nor, in our opinion, has it any clinical basis.

For the reasons given in the preceding pages, we now consider ourselves justified in offering the following classification, in which we distinguish three species of malarial fever, differing from each other in their clinical features and produced by different species of parasites:

1. Quartan infection, which includes (*a*) quartan; (*b*) double quartan; (*c*) triple quartan, that is to say, quotidian of quartan origin; (*d*) some irregular and subcontinuous fevers.

2. Tertian infection, which includes (*a*) tertian, (*b*) double tertian or quotidian of tertian origin, (*c*) some irregular and subcontinuous fevers and long-interval fevers.

3. Estivoautumnal infection, which includes (*a*) the estivoautumnal tertian or malignant fever, which is the dominant clinical type; (*b*) some quotidian fevers; (*c*) many irregular fevers, nearly all the subcontinuous (remittent) or continued fevers, and the greater number of the so-called long-interval fevers.

To this last variety of malaria belong all the pernicious and a large part of the tropical fevers. As we know, other varieties, especially the tertian, have been observed in the tropics; but if we wish to preserve the widely used name of *tropical fever*, we must limit the term to the dominating variety, which is the kind described by us as the estivoautumnal.

The classification given above takes no account of presumed results from theories still under discussion, but is based upon the best-established facts of parasitology, epidemiology, and clinical experience.

As a natural sequence to what has been stated in reference to the

classification of parasites, these three varieties of fevers may be positively considered to be produced by three distinct species of parasite. In fact, the parasites of the quartan, the tertian, and the estival variety show: (1) Perfect constancy in their morphological and essential biological characteristics, to the extent that they may always be easily recognized on microscopical examination; in all malarial countries of the world they have been found, and the same fundamental characteristics have been recognized in them; (2) an intimate and indissoluble relation in each determined clinical species; (3) the possibility of inoculation from man to man, with reproduction of the typical clinical and parasitic forms; and (4) the impossibility of transforming one type into another.

In the second place, from a clinical point of view, the three varieties of malaria are so clearly distinct that not only the typical but also many of the complex and irregular forms may often be diagnosed by the physician without having recourse to an examination of the blood. Finally, epidemiology demonstrates that there are places and seasons in which these various species of malaria are, so to speak, almost isolated. Thus, there are places and seasons in which the quartan dominates, in others the ordinary tertian, and in others again the malignant tertian, which is the principal clinical form of the estivo-autumnal group. It will be understood, of course, that we refer to the predominance of one species of malaria over another, and not to the exclusive domination of any one type, although in some special cases, as we shall see later, even this may occur.

A few words remain to be said upon the group of estivoautumnal fevers. As we have seen, every variety of malaria corresponds to a fundamental clinical type of periodic intermittent fever, around which are grouped the complex and irregular clinical forms. Only in the third variety we have two fundamental clinical types, the *summer tertian* and the *quotidian*. The question as to whether these two types are fundamentally distinct must be examined into by clinical as well as by parasitical researches. From a clinical point of view, we may consider it as an established fact that an estival quotidian form does exist, although it is rarely manifested in a regular manner. After the description first given by Marchiafava and Celli of this variety, a quotidian due to the small estival parasites was seen by Mannaberg, Ziehmann, Feletti, and others. From the cases noted by us in all malarial seasons, following our first published work on the subject, we have learned that the type of tertian called by us the malignant is the most important and by far the most frequent; we have also learned that nearly if not quite all the pernicious fevers are derived from this tertian. In this connection it is well to recall what we have already

written upon this subject, namely, that the estivoautumnal endemic does not occur each year with characteristics which are absolutely identical; for instance, the cases of malarial infection with little melanæmia, and with abundant fission of parasites without a trace of pigmentation, which were first observed in 1885 by Marchiafava and Celli, have never since then been seen by us. This fact leads us to believe that even in the predominant clinical types there may exist a certain amount of variability, a circumstance which should be borne in mind by investigators who are endeavoring to obtain reliable data in any one malarial region or in one or a few special seasons. We may add that recent observations have shown us that some quotidian fevers should be considered *mild double summer tertian with short attacks*. Thus a summer tertian when it becomes attenuated and tends to a spontaneous cure (which does occur, although rarely), may assume the clinical form of a quotidian fever.

The question of the special parasites has already been discussed in a previous section. In our first published works upon the subject we held it to be probable that the estivoautumnal species of parasite included two intimately related varieties, but that while one of them almost completed its development within twenty-four hours, which would explain the summer quotidian, the other completed its cycle in forty-eight hours, causing the malignant tertian. It is to be noted that in this relation we did not speak of species, but of varieties. We expressed ourselves with much reserve, the reasons for which we have given elsewhere. In our classification of fevers, in order to avoid the introduction of matters still under discussion, we did not divide the third species of malarial fevers into two sub-species, for this would have been to admit the existence of two varieties of parasites (whether of distinct types or, as we have stated, *intimately related*), and we limited ourselves to a clinical distinction of the two fundamental types, as it seemed preferable to remain upon clinical ground while waiting for the parasitic problem to be resolved.

While still maintaining this reserve, if we were to express an opinion upon the subject, we should say, in spite of the objections made by some authorities, that the proposed subdivision of the fevers and the estivoautumnal parasites is perfectly admissible. We are led to the same conclusion by the knowledge that the very same estivoautumnal fevers are met with in the various malarial regions (with some differences in their course and in their type), a matter which has been proved beyond a doubt. To give an example: the differences between the estivoautumnal fevers of the Roman Campagna and those described by Feletti in the plain of Catania are very evident. Feletti gives the following description of these fevers: "As a

rule, the fevers due to the Laverania malariæ are first manifested in a subcontinuous, or a subintrant, or a quotidian form. But even in the quotidian variety the attacks show some irregularities, and for the most part are prolonged in duration, . . ." etc. And he adds that, while these fevers are most obstinate, they do not become pernicious.

These clinical characteristics are seen to be quite different from those which we described for the estivoautumnal tertian. We have already given the reasons why we could not accept the opinion of Grassi and Feletti, that the parasite of malaria which they had studied (Laverania malariæ) belongs to a different genus from the one which we studied in Rome. Indeed, the result of all our researches has been to establish the certitude that the parasite of the estival tertian has a phase of its existence characterized by crescent bodies, and to disprove the assertion that our cases were due to a mixed infection.

And yet it seems to us that there must be some reason for the divergences. In all probability it is the following: In the estival tertian, and in the subcontinuous, subintrant, and quotidian fevers respectively, as observed in Catania, we have two varieties of parasites which possess much mutual affinity: in their earlier form and in the crescent phase they are identical, the only difference being that in the second variety, during the height of an attack, we do not find in blood taken from the finger those large, pulpous, pigmented bodies which we see in our estival tertian. The clinical differences, however, are noteworthy; on the one hand we have a special tertian, on the other a quotidian, irregular, or subintrant fever, etc. In our regions where the tertian dominates, there is a much greater frequency of pernicious fevers.

Although the parasitical differences are so light, the differences in the clinical forms seem to us to justify the distinction of two varieties of parasites, the one giving irregular fevers or, if they tend to take on any type, the quotidian; the other giving fevers of which the tertian is the type.

We have even observed here in Rome a type of fever with a course similar to that decribed in Catania, whence our first division of estival fevers in their typical form into quotidian and tertian. The fact that one type may predominate in one region and a second in another strengthens us in the maintaining of this distinction.

It is necessary always to begin by making distinctions, and it is also the best method of study. Should our further researches bring the conviction that the estivoautumnal parasite is always and everywhere identical, like that of the tertian and the quartan, we would, of

course, give up this subdivision. For the present it seems to us that many facts support the theory that the estivoautumnal species of parasite includes several closely related varieties.

Quartan Fever.

History.—The ancient physicians have bequeathed to us a complete clinical description of quartan fevers, to which recent observers have been able to add nothing but the exact description of the complicated and irregular clinical varieties of quartan infections, which could not have been clearly recognized and described from a mere study of the course of the disease by one without knowledge of the quartan parasite. To modern researches is also due the theory of mixed malarial infections.

The ancients, moreover, described a double and a triple quartan; and in addition a duplicate and a triplicate quartan, in which there were respectively two and three febrile attacks in one day, followed by two days of apyrexia, and then by two and three febrile attacks on the fourth day. These varieties we have never observed, nor do we know of any one in modern times who has seen them.

Hippocrates considered the quartan to be the least dangerous of all the fevers—"omnium febrium tutissima." Relatively recent clinicians, as Borsieri, have described malignant and even fatal forms of quartan. These varieties are unknown to the modern physician, but we do know several morbid sequelæ, as dropsy and œdema, and some complications, such as diseases of the chest, which were described in detail by Borsieri.

We must look to the writings of the present day to obtain any positive knowledge as to the etiology and pathology of quartan fever; these are due to the researches of Golgi, who was the first to describe the parasite of the quartan, and to teach us how to distinguish it from that of the tertian, and who followed its evolution in relation to the development and the successive occurrences of the febrile attacks.

The quartan is produced by the development in the blood of the quartan parasite (amœba febris quartanæ, of Golgi; hæmamœba malariæ, of Grassi), which completes the cycle of its existence in about seventy-two hours. The infection may be manifested in various clinical forms, which are perfectly explained by a study of the parasites. These forms are: (1) Simple quartan fever, (2) double quartan, (3) triple quartan, and (4) some irregular and subcontinuous fevers of quartan origin.

Distribution.—Quartan infection, with the more complicated fevers which parasitology shows to be due to it, appears to be less widely

distributed than the other forms of malaria. It is, to be sure, found in all malarial countries, but while in regions of mild types of malaria it is less frequent than the ordinary tertian, in the regions of severe types of malaria it is much less frequent than the ordinary tertian and the estivoautumnal fevers. In Germany, where the types of malaria are the mildest, the quartan as a rule is not found; it was recognized by Wenzel (quoted by Ziemann) during the extensive epidemics of malaria at Wilhelmshaven which developed when the harbor was improved.

In the United States it is not of common occurrence; Thayer and Hewetson saw only fifteen cases (in Baltimore) out of sixteen hundred and eighty cases of malaria. Dock, during the course of three years, saw only one case in Texas. In the tropics it would appear to be still more rare. R. Koch observed only one case in tropical Africa, and Ziemann found none in Kamerun. These facts are quite in harmony with the statement of Sternberg that the quartan is almost unknown in tropical regions. Even in India this type of fever has been met with but rarely, as we learn from the experiences of Dr. Crombie in Calcutta and its neighborhood; on the other hand, according to Ross, in Madras and in other parts of India the quartan parasite is not so rare.

Still it is perfectly correct to assert that the quartan is a fever of temperate climates. In Italy it has been observed in all malarial regions, but with particular frequency in special localities, as in the neighborhood of Pavia, etc. It would appear, therefore, that the quartan occurs with great rarity in the regions of mild malaria and in hot countries, while it is more frequent in countries which hold an intermediate position as to the intensity of the infection.

There are certain localities which are known to be centres, as it were, for the production of this fever. Authors quote in this connection the classical observation of Trousseau, who long ago, in giving his views upon the various kinds of malarial fever, said: "The febrile types would appear to depend more largely upon the nature of the miasm, and especially upon the locality in which the infection occurs, than upon the condition of the individual who is affected. Tours and Samur, which are both situated upon the left bank of the Loire, would seem to be under the same climatic and telluric influences, yet in Tours we find only the tertian variety, while all the quartan cases which I have seen were in persons coming from Samur, from Rochefort, or from some other region. One of the facts which made the deepest impression upon me was the following: Fourteen soldiers from Samur came to Tours to testify at a court-martial; they had scarcely been ten days in the latter city when nine of them were

obliged to go to the hospital suffering from quartan fever, the germs of which they had certainly contracted in Samur, since all the fevers observed in Tours and its environs were of the tertian type."

We have already called attention to the fact that this type of fever does not occur with the same frequency at all seasons of the year. In Rome and its neighborhood the quartan is most frequent in the autumn and in the winter: we are unable to give exact figures, because of the absence of statistical observations repeated for several years. In Catania, according to Feletti, it is more frequent in the summer and in the autumn than in the spring. In Lombardy it is well known to the people that the quartan occurs more frequently in the autumn. We cannot, however, deny the fact that the quartan fevers which are made manifest in the winter and in the autumn are due to an infection taken towards the end of the hot season, having a long latent period.

Simple Quartan.

The simple quartan fever is characterized by febrile attacks which are usually of short duration (eight to ten hours) and which follow each other regularly, separated by a two days' interval of apyrexia. The simple quartan may be so primarily, the febrile attacks returning every fourth day from the very beginning. Often, however, the fever is irregular at the onset, and only in its relapses becomes regularly quartan; or the infection may begin as a triple or double quartan, becoming simple in time, with the relapses.

It is the most regular of all the varieties of malarial fever, and it is quite natural that in this rather than in any other type Golgi should have discovered the intimate relation which exists between the clinical manifestations and the changes in the life of the hæmatozoon. Perfect regularity in the succession of the attacks may continue for as long as several months. But it may also happen that several attacks will anticipate or delay their coming by several hours; in this case we speak of an anticipated or a postponed quartan. We have often seen a retardation in the attacks with spontaneous recovery occurring later.

The febrile attack is attended with the same symptoms in the quartan as in the tertian. All writers agree in distinguishing three stages: (*a*) the cold stage, or that of the chill, (*b*) the hot stage, (*c*) the stage of defervescence, or sweating.

The Cold Stage.—The attack may begin abruptly without prodromes, the patient being apparently perfectly well; as a rule, however, there are vague premonitory symptoms, consisting in general discomfort, headache, yawning, and a feeling of exhaustion, some-

times nausea and vomiting. Following these sensations is the feeling of cold, which begins at the extremities and then invades the body; or, on the other hand, it may begin by horripilation which the patients refer to the back—the sensations rapidly increasing until the chill occurs. While in the other forms of fever the chill may be absent or be abortive in type, in the quartan it is intense and prolonged and, as all physicians can testify, is not apt to be absent. In the first attacks the chill is usually less intense than in the succeeding ones. We have noted its absence in a few rare cases, as for instance in old people and in sufferers from chronic malaria who had had many attacks of fever. It may also be absent in children.

This is the part of the attack in which the suffering is the most intense. The lips become blue, the extremities cold and cyanotic, the skin is in a condition of goose-flesh; the pulse is weak, rapid, and sometimes irregular; the respirations are quick and short.

Frequently there is vomiting of food or drink stained with bile, and often there is diarrhœa. The headache is apt to be intense; the whole body shakes with the chill. The patient doubles himself up and rolls himself in the blankets even when the surrounding temperature is high, and derives comfort from hot applications. The pupils as a rule are enlarged, and there may be some visual disturbances.

We have said that the skin becomes cold and cyanosed, especially in the extremities, but the intense and most painful sensation of cold is not in absolute proportion to the low temperature of the periphery. As to the internal temperature, not only does it rise notably and rapidly during the chill, but as a rule the pyrexia precedes the chill, usually by a half-hour or an hour, but sometimes by three or four hours. At the beginning of the chilly sensations it is not unusual to find a temperature rising to between 38° and 39° C. (100.4° to 102.2° F.) or even to between 39° and 40° C. (102.2° to 104° F.). In the cases in which the chill is delayed for several hours after the onset of the fever, the patients themselves state that the fever was first *hot* and then *cold*.

The duration of the cold stage may vary from a quarter of an hour to half an hour or more; in some rare cases it is very short and in others as long as an hour.

The Hot Stage.—The chill fairly over, the patient continues to have transitory sensations of cold interrupted by flashes of heat which invade the whole body. The sensation of heat then becomes intense and continuous; the skin is dry and hot, the conjunctivæ are injected, the respiration is rapid, and the pulse is frequent and often dicrotic. Sometimes there is a dry cough, sometimes we note vomiting and diarrhœa. As a usual thing in the ordinary febrile attacks of the ter-

tian and the quartan types, there are no marked nervous symptoms; the patients are generally agitated and even anxious, but in some cases they are calm and show a tendency to drowsiness. The suffering comes from an oppressive sense of heat and from headache. The results obtained by a physical examination are variable, the color of the skin, the size of the spleen, etc. depending upon whether the infection is of recent or of ancient date. A careful examination will usually reveal enlargement of the spleen even in a first attack.

The temperature reaches its height during this stage, and as a rule stays there but a short time, the greater number of typical quartan attacks (and the same may be said of the tertian) showing in the graphic curve only one undulation; in other cases again, when the elevation of temperature is of longer duration, the curves (if the temperature is frequently taken, say every two hours) will show several oscillations and two undulations, one at the beginning and one preceding the crisis.

When we add that during the attack it is not infrequent to find various skin eruptions, such as an erythematous rash or more often eruptions like urticaria or measles which disappear with the fever, we shall have enumerated the chief symptoms which have been observed by various writers during the ordinary intermittent fevers—symptoms which the physician has occasion to observe every day. .

The Stage of Sweating.—When, after a certain number of hours—four, five, and often more—of heat, the first drops of sweat appear upon the forehead and the chest, the patient at once experiences a feeling of relief and of well-being. The sweat rapidly increases, becoming usually very profuse, and at the same time the temperature is lowered, the pulse diminishes in rapidity so as to become slow and full, and the respirations become normal. In from two to four or five hours the temperature descends to normal or even to subnormal, and may remain so during the whole stage of apyrexia. At the end of this stage it is not unusual to find the pulse beating only 40 or 50 times a minute. The patient falls into a refreshing sleep.

We have already spoken of the alterations in the urinary secretion during a classical attack (see page 217).

The average duration of the whole paroxysm in its three stages is usually from eight to ten hours in the quartan; in the tertian it is apt to be a little longer. But there are prolonged as well as abortive attacks, in the latter of which the phenomena of the first and the third stages are scarcely perceptible.

As regards the *prolonged attacks*, we have scarcely ever seen any. In some exceptional cases the attack may last more than twenty-four hours; the temperature curve then shows two chief undulations, one

at the beginning and the other precritical, separated by a notable remission, although the temperature still remains above the normal; this may give the impression that we have to do with subintrant attacks.

We have already said that in these classical forms of intermittent fever, marked nervous symptoms are absent in the adult. This is not the case, however, in children. Physicians have long noticed that the stages of cold and of sweating are often entirely absent in patients in the early years of life. During the period of invasion of the fever, for the most part we observe only a certain amount of cyanosis of the mucous membranes and of the extremities, with a lowering of the temperature in the latter; and sometimes a general convulsive attack takes the place of the chill, beginning by slight movements of the eyelids and the eyes, or of the extremities, and it may be severe.

The quartan attacks may begin in the morning, or at noon, or shortly after, but as a rule, they develop in the afternoon; very rarely they occur at night. The patients then have two days of apyrexia, during which time the sense of well-being is complete, so much so that they can attend to their usual occupations; not infrequently during the whole intermission the temperature remains subnormal and the pulse rather slow. This, of course, in the pure quartan types only.

Biological Cycle of the Quartan Parasite.

We have already discussed the subject of the biology and the structure of the quartan parasite. In this connection we will merely speak of the chief facts concerning the cyclic development of the parasite in relation to the periodic development of the fever. Every one now appreciates the importance of an examination of the blood for an exact diagnosis of the nature of the fever. Indeed, not only does it enable us to determine whether we have to do with a simple or a double quartan, and to ascertain the quartanary origin of a quotidian fever, but it also makes it possible to foresee a coming attack, and to diagnose the origin of an irregular type of fever.

If we examine the blood of a patient suffering from quartan fever on the morning of the first day of apyrexia, we shall find within the red corpuscles young amœbæ as large as a sixth or a fifth of the corpuscle itself, with granules of pigment at their periphery, hyaline of aspect, and endowed with torpid movements, as shown by the alterations in their contour. The red corpuscles which contain them are of the normal size and appearance. During the whole period of apyrexia, the amœbæ, while retaining their usual appearance, gradually increase in size; the pigmentation at their periphery becomes more

abundant, and their development continues until they are almost equal in size to the red corpuscle which has become reduced to a mere halo around the parasitic body. Six, eight, or ten hours before a new attack begins certain changes occur in the amœba, leading to segmentation (sporulation).

At this period of development the parasite has grown so large as to fill up nearly all the red corpuscle, the peripheral portion of which forms a sort of membrane around the amœba. Some of these bodies in the adult state are apparently free, and their pigment is no longer at the periphery but is irregularly distributed. Hence, when the process of fission begins, the pigment is apt to arrange itself in radiating bands, tending to meet at the centre; and while this concentration of pigment goes on until there is formed a central accumulation of black granules or one mass, the substance of the parasite shows the striations more and more plainly. The result of all this is the formation of from eight to ten small oval or round bodies which are arranged like the petals of a daisy around a central mass of pigment.

This segmentation (sporulation) is completed a little before or is coincident with the onset of the febrile attack. The fever once developed, the segmenting bodies disappear, and within the red corpuscles we see young unpigmented amœbæ endowed with amœboid movements, which in their turn slowly develop and become pigmented, thus repeating the cycle of existence already described. The process of segmentation may occur in a less regular manner; the pigment instead of being gathered together in one central mass may be in several smaller accumulations, or it may remain scattered between the spores, etc. Sporulation may take place before the amœba has attained the volume of the red corpuscle, with at most six to eight spores, as seen by Golgi and Antolisei. But these cases are quite exceptional. Fission forms may be found seven or eight hours previous to the febrile attack, and some young parasites may appear two hours before the fever has begun, to go on increasing while the fever is present. These facts, however, do not invalidate the law of Golgi, that "the onset of each attack coincides with the maturation of a group or generation of parasites."

In addition to the mature bodies which reach the stage of fission, we may find in the blood of patients suffering from quartan fever, several hours before the attack (or the previous day), free pigmented spherical bodies similar to those of tertian, from which they differ only in having an immobile or slightly mobile pigment, and a more refractive protoplasm. We may also find spherical bodies as large as, or larger than, the red corpuscles, usually with an immobile or slightly mobile pigment, but sometimes with pigment in active mo-

tion, which ends by breaking up into little hyaline globules. Bignami and Bastianelli and others have held these bodies to be sterile forms of the parasite which disintegrate in the plasma. According to our experience it is rare to find *flagellated* bodies in the quartan type of fever, but further research is necessary in this field.

The cycle of existence of a quartan parasite is completed in the circulating blood, so that all its phases are readily open to observation. Bastianelli and Bignami, in a study of blood extracted during life from the spleen, did not find any notable differences in the distribution of the parasitic bodies compared with that in blood extracted at the same time from the finger (the differences are very marked in the estival type); but they found in the spleen a greater number of pigmented leucocytes, or leucocytes enclosing parasites, or entire bodies in fission, or masses of free pigment representing the residua of sporulation.

The inclusion within the white corpuscles of a certain number of bodies in fission occurs correspondently to every febrile attack (Golgi). This fact, and the one ascertained by Bastianelli and Bignami, that not all adult bodies undergo sporulation, but that some are free in the blood and die (although this occurs much less frequently than in the tertian), explain why the red corpuscles which are invaded by the young amœbæ during a febrile attack are never so numerous as we should expect to find them, considering the number of adult bodies and bodies in fission that are present.

The question as to whether any relation exists between the number of the parasites and the severity of the fever has been under discussion. Although, as has been stated, in the quartan the entire life of the parasite is developed in the circulating blood, so that it is easily accessible to observation, yet it is natural that the replies to the question should differ. It is not a simple matter to estimate the number of the parasites. Moreover, it is very likely that differences exist even in the same subject according to whether the attack is a primary one or a relapse; in the relapses it appears that the presence of large numbers of parasites causes less severe attacks than in the primary infections. In relapsing quartans we have even noted the presence of spores without any fever being present. Finally, it is easy to understand that there should be differences in different individuals, and it is not to be expected that all should react in the same way to a given number of parasites. With these reservations, we may admit in general that it is not to be denied that there is a certain relation between the number of the parasites and the severity of the fever. According to Antolisei the relationship is not between the number of parasites present in the apyretic stage and the next attack, but between the

number of spores and the coming fever. To us it seems that this relationship would be more clear and easier of demonstration if we limited our study to the various attacks in one person; for instance, in a patient with double and triple quartan, by prolonged and careful observation and by noting the various parasitic groups and the number of the bodies in their various stages of development, we can within certain limits predict whether an attack will be slight or severe.

Double Quartan.

Two febrile attacks occur on two successive days, followed by a day of complete apyrexia, after which come the two distinct attacks on two days again. This type of fever may possess these characteristics from the beginning, or a quartan may become double. In some cases the two consecutive attacks are of about the same intensity and duration; in others a light and short attack may be observed to follow a more intense and prolonged one with a certain amount of regularity, so that the slight and the severe attacks correspond regularly to every fourth day.

In an examination of the blood we find two groups (or generations) of parasites which with regularity mature and multiply with about a

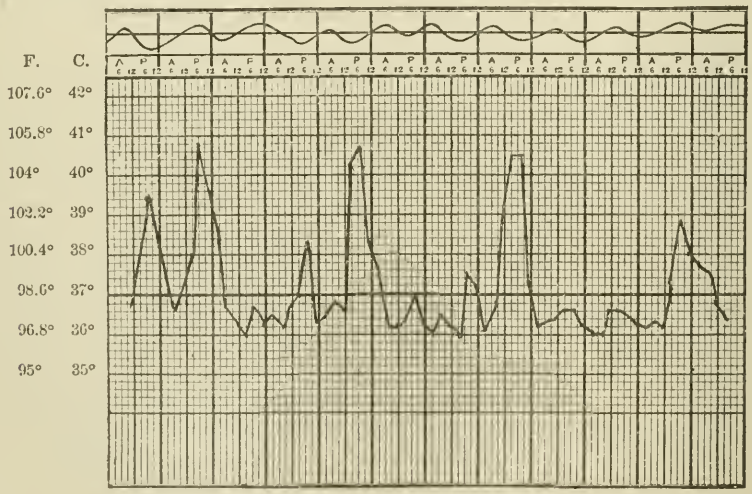


Chart No. 1.—Double Quartan, Becoming Single.

day's interval. This succession of events, if not interfered with by treatment, may continue for months. In a case described by Golgi the double quartan lasted two months.

In the cases in which the double quartan becomes simple (see

chart No. 1) one may observe, as we have several times done, that a generation of parasites will diminish with some rapidity; then on the day when an attack is expected, in spite of the presence in the

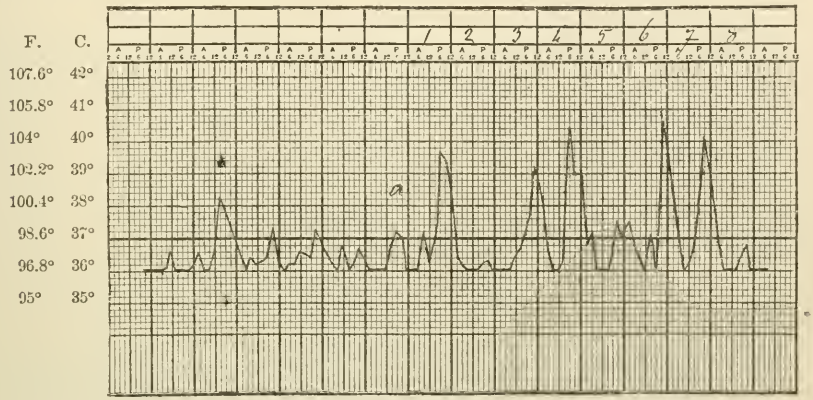


Chart No. 2.—Double Quartan. At *a* were found a few quartan forms in fission, unmarked by any febrile access.

blood of some fission forms, the fever is not produced, or there is merely a slight elevation of temperature; then the whole group of parasites disappears. Nevertheless, the double quartan may reappear during a relapse.

On the other hand, in cases in which a simple quartan becomes double, we may suppose that from the beginning there exists a group of parasites too scanty in number to cause the febrile attack, but gradually increasing until the necessary amount of energy is acquired. This cannot always be proved by an examination of the blood, but in a case which was under our observation we found a very small group of parasites, which reached maturity and multiplied on a day of apyrexia, increased in the next cycle and caused fever (see chart No. 2).

Triple Quartan.

Three distinct groups of quartan parasites come to maturity and multiply on successive days. As a result we have a quotidian fever whose origin from the quartan may be demonstrated by an examination of the blood. This origin of the quotidian fever may be diagnosed by merely clinical observation, without examination of the blood, when it follows a simple or double quartan, or when, on the contrary, the quotidian fever becomes a double or a simple quartan by the suppression of one or two attacks (see chart No. 3). The suppression may occur spontaneously, or it may be artificially produced by means of quinine; this remedy given in small doses a few hours

before an attack may suppress one group only of parasites without so injuring the others as to interfere with their development. In this manner, as physicians have long observed, the fundamental type becomes manifest.

It may happen that the individual attacks resemble each other absolutely as to the hour of invasion, the height of the temperature, etc. In other cases, however, the hour of the invasion and the severity of the successive attacks vary, and each attack will, up to a certain point, correspond to the one following it three days later. Although this is of rare occurrence, yet in our experience it is sufficiently marked to allow of a diagnosis of the quartan origin of a quotidian fever; we must bear in mind, however, that even in a pure quartan

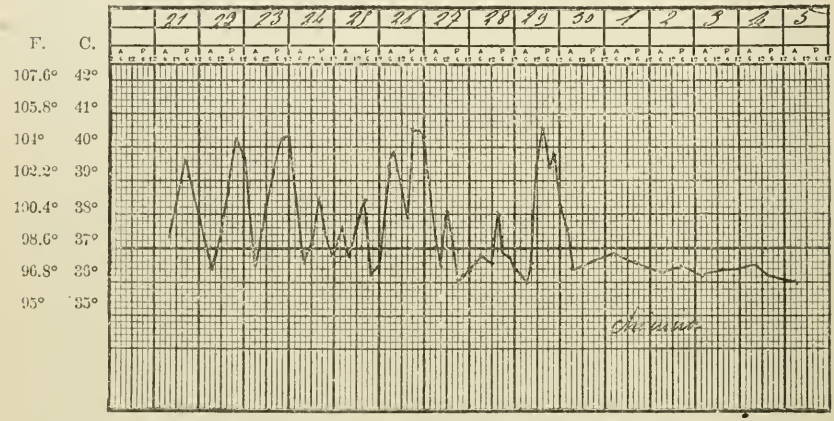


Chart No. 3.—Triple Quartan Tending to Become Single Spontaneously.

the attacks may delay or anticipate or be of different degrees of intensity.

For the most part the single attacks of a quotidian of quartan origin are clearly distinct, and separated by an interval of subnormal or normal temperature. But two successive attacks may, on the other hand, be *subintrant*, the chill of one attack seizing the patient before the previous attack has quite ended in the sweating stage.

Finally, the quartan may from the onset be manifested as a quotidian which in the course of time becomes modified. This has also been noted by Feletti in a case observed in Catania. In our opinion, it explains why in the relapses a pure quartan not infrequently becomes double or triple; the probable reason of this is found in the fact that at the beginning three generations of parasites are found in the blood, of which some become so attenuated that they are incapable of causing fever, while others become strengthened; thus it hap-

pens that apparently different febrile types follow each other in the relapses.

An examination of the blood, as we have stated, demonstrates the presence of three distinct groups of parasites. As a rule, the observer is in the beginning puzzled by finding parasites in various stages of development which it is difficult to unite into groups; usually, however, a careful and prolonged examination will enable us to differentiate the three generations, and also to prognosticate the triple quartan. We may even differentiate one of the groups, although the parasites composing it be in too small numbers to produce an attack on the following day, and we may perhaps follow it for some weeks before it becomes strong enough to occasion the disturbance. Such exactness of results in the examination of the blood is due, as Thayer also observes, to the fact that the quartan parasite completes its whole cycle of development in the circulating blood, so that no phase of its existence escapes a prolonged examination.

The spontaneous transformation of the triple into a simple quartan may occur from progressive attenuation of two attacks and respectively of two groups of parasites. In some cases it is noticed that while two attacks become milder, the residual attack becomes more intense, as if the increase in pathogenic energy of a group of parasites were in relation to the attenuation of the other two (see chart No. 3). We have not, however, sufficient data to warrant the assertion that there is any causal connection between the two facts, viz., the increase of one parasitic group and the simultaneous diminution of one or of two other groups.

Irregular and Subcontinuous Fevers of Quartan Origin.

The first are rare, and the second still more rare. We have seen a few cases of irregular intermittent fevers, but not one of continuous fever. Two cases of subcontinuous (remittent) fever were seen in Rome by Antolisei; both were primary infections and occurred in the autumn. Following in them the development of the quartan parasite, Antolisei noted the presence of small fission bodies with a few granules of pigment and six to eight spores, taking up scarcely a third of the red corpuscles, and he interpreted the fact as denoting that a cycle of development was hastened by the quartan parasite. In these cases, owing to the presence in the blood of several generations of parasites, we may see all the stages of existence of the hæmatozoon in one and the same preparation. Feletti also speaks of irregular and subcontinuous fevers of quartan origin observed especially at the beginning of the disease.

Clinical Course.

The clinical course of the quartan may vary greatly, as indeed would follow naturally from what has already been said. We have stated that from the beginning the fever is often irregular and may even suggest a continuous or a quotidian type (see chart No. 4), and only later, either spontaneously or through the action of a specific remedy, does it become a double or simple quartan. As every one knows, it is a more obstinate type of fever than the tertian; it is not unusual to see a quartan which began in the autumn, relapse in spite of all remedies through an entire winter, to become exhausted in the

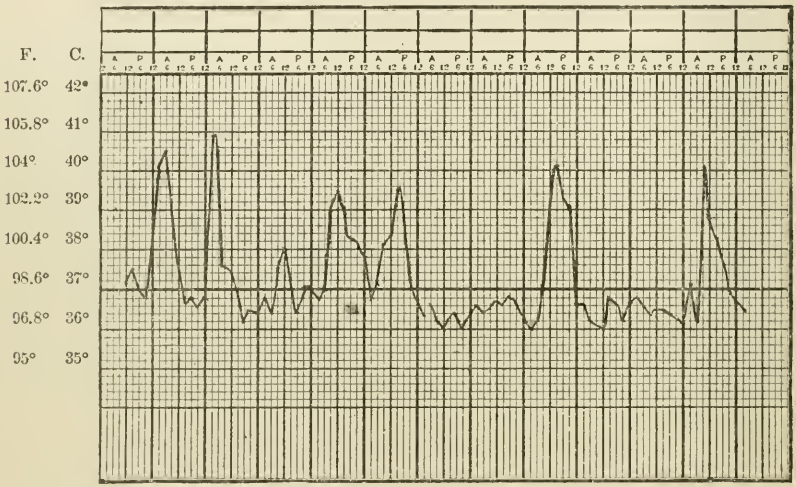


Chart No. 4.—Irregular Triple Quartan, Becoming Single Spontaneously.

spring. It has even been asserted that in some rare cases it continues for several years. A characteristic of the quartan is that it may continue in a monotonous course for several months, the attacks being more or less similar. In fact, the parasites show no tendency to increase in number and to accumulate, as is the case in the estival fevers which tend to become pernicious, but many of them are incompletely developed. It has been noticed, for instance, that the number of young parasites found in the blood at the end of an attack is always less than the observer expects to find, who remembers the number of fission bodies seen before the attack. Thus it happens that the stock of parasites tends for a certain length of time to remain constant, but gradually becomes less, and the fever then ceases spontaneously; after an interval of variable length, however, relapses occur.

We observe the spontaneous cure of the quartan more rarely than that of the tertian, and are more frequently obliged to resort to therapeutic measures to combat its persistency. When spontaneously cured there is usually a gradual diminution in the intensity of the attacks, and a corresponding diminution in the number of parasites in the blood is noted, until they finally disappear.

So far as the *prognosis* is concerned, we must remember that a quartan infection does not give rise to grave symptoms (pernicious malaria) even when the fever is of the subcontinuous or subintrant type, and the parasites are found in great abundance in the blood, or when it occurs in debilitated or aged subjects. We may add, however, that the anæmia which follows an obstinate relapsing quartan may be very marked, like that which follows other varieties of malaria, and that chronic invalidism may follow quartan infection alone. But all this can be avoided by appropriate medication.

A mixed infection of quartan with other varieties of malaria may occur. Thus we have not infrequently observed cases of mixed quartan and tertian, and even of quartan and estival. There is an intermittent, irregular fever, which sometimes shows a tendency to become continuous. But it may also happen that in these mixed infections the parasites will alternate; thus in one patient we noticed a diminution in the intensity of the quartan attacks together with a diminution in the number of the parasites, while little by little tertian bodies began to accumulate which gave rise to quotidian attacks.

Complications.

Complications with other diseases have rarely been observed. We recall to mind the case of a paraplegic individual with cystitis and pyelonephritis in whom a relapse of quartan fever occurred in the last weeks of his life, and followed its typical course. In one case of quartan and pneumonia observed by Antolisei, the pulmonary affection completed its natural course without being in the least degree influenced by the coexistent quartan. It is worthy of note that the quartan parasites diminished to a marked extent during the course of the pneumonia.

Sequelæ.

Morbid sequelæ may occur not only after fevers of a grave type (which is the rule), but also after the quartan and the ordinary tertian. Nervous phenomena as sequelæ of the quartan appear to be entirely absent. Alterations in the urinary excretion or actual renal disease may be found after and even during the course of a mild malarial infection; such are polyuria, albuminuria, and even ne-

phritis. But it may be asked whether they are due to the malaria or to complicating causes.

In one case we observed the development of progressive anæmia in a woman who had for a long time been suffering from quartan fever; it became aggravated and took on the type and symptoms of a pernicious anæmia without ever at any time, even up to the fatal issue, showing any relation to the malarial infection. We can, of course, not deny that the quartan affection had some causal importance in the production of the progressive anæmia, but it is evident that we must admit the existence of other etiological factors whose exact influence we do not know.

Tertian Fever.

History.—The older physicians, as for instance Torti in his classical work, as a rule included the greater number of pernicious fevers under the head of the tertian. Clinical observation, however, had demonstrated to them the existence of two forms of tertian; they called one *tertiana legitima, sive exquisita, sive pura*, and the other *tertiana notha, sive spuria, sive extensa.* The first corresponded to that which we call the ordinary tertian, the subject of this discussion. The second was less clearly described in its clinical aspects by the older physicians than the first; the attacks are longer than those of the tertiana legitima, and the prognosis is graver, as pernicious symptoms may develop (Borsieri). The best and most clearly given clinical description and distinction between these two forms of tertian is that found in the works of Celsus, quoted by us in relation to the estival tertian.

The sharp distinction made by the older writers is not mentioned by recent writers, so far as we know, and up to a few years ago it had been so completely forgotten by physicians that they did not know how to reconcile the statement of the modern scientists that the majority of the pernicious fevers are derived from the quotidian, remittent, or continuous fevers in the beginning, with the old opinion that nearly all the severe fevers are derived from the tertian.

The question has been completely answered only by our recent researches which have demonstrated the existence of two varieties of tertian fever, clinically distinguished by thermometrical tracings, and produced by two distinct species of malarial parasites—the ordinary tertian and the malignant or estivoautumnal tertian. We are here concerned only with the first.

Classical medicine has already distinguished several clinical types in this tertian: the *simple* and the *double* tertian, and in addition the

duplicate and the *triplicate* tertian. In the duplicate tertian there are two febrile attacks in one day, then an intervening day of apyrexia, with two distinct febrile attacks on the third day. In the triplicate tertian there are two attacks in one day, as in the preceding variety, one attack on the second day, two attacks on the third day, and so on. Of these forms we personally know nothing.

As for the quartan, so for the tertian, our knowledge of the etiology dates from the publication of the works of Golgi, who described its parasite, showing us how to distinguish it from the other malarial parasites, and followed its cycle of development in relation to the succession of the febrile attacks.

What we call the *ordinary* tertian, to distinguish it from the estivoautumnal variety, is produced by the development in the blood of the *tertian parasite* (Amœba febris tertianæ, of Golgi; Hæmamœba vivax, of Grassi), which completes its entire life cycle in about forty-eight hours. As this parasite may give rise to fevers of apparently varying types according to whether we find one or two groups of hæmatamœbæ in the blood, and according to whether the single groups develop in a regular or an irregular manner, we believe that we are justified in speaking of a tertian infection which may be manifested in several clinical forms. These are: (1) Simple tertian fever, (2) double tertian or quotidian of tertian origin, and (3) some irregular and subcontinuous fevers.

Distribution.—Tertian infection, or the classical tertian and the more complicated fevers to which it gives rise, is the most generally distributed of all the varieties of malaria. It is found in all malarial countries. In countries where the malaria is of the mildest type, as for example in Germany, nearly all the cases of the disease belong to the tertian variety. In regions where malaria is moderately mild, as in Lombardy, it dominates together with the quartan. In the countries with temperate climate, where grave malarial fevers prevail, as for instance, the Roman Campagna, ordinary tertian dominates at the beginning of the malarial season, that is to say, in the spring; then, in frequency and in importance it yields to the other variety of tertian, the estivoautumnal. But even in the summer and in the autumn the cases of tertian and their complications are still numerous, and are seen in their pure type, or complicated with the estivoautumnal. In the province of Catania, according to Feletti, the tertian is more easily taken in the summer and in the autumn than in the spring. This variety of malaria has been seen in all tropical countries, while the quartan is comparatively rare, and in some regions may be altogether absent. In the German colony of East Africa, the ordinary tertian, according to R. Koch, constitutes ten per cent. of all the cases of

malaria; the remining ninety per cent. being given to another species of malaria which in nowise differs from our estivoautumnal tertian.

Researches have been made to ascertain whether, in regions where several races live together, any perceptible influence is exercised by *race* upon the nature of the infection.

Thayer and Hewetson observed in Baltimore 27 cases of malaria in negroes. According to their statistics, in 59.2 per cent. of the cases the fevers were of the tertian variety, and in 33.3 per cent. of the estivoautumnal; in white men 62.5 per cent. of the cases were of tertian infection, and 34.7 per cent. of the estivoautumnal. It would appear, therefore, from these figures that there is no essential difference in the susceptibility of the white and of the colored race to these two varieties of malaria. We find, however, that the negro suffers more frequently from the simple tertian infection than he does from the double tertian, the sufferers from the latter being predominantly members of the white races.

The observations of L. Martin in Sumatra would lead us to a different conclusion. He noted that while the Europeans nearly all become affected by grave forms of the disease, the Malays and Javanese, who are relatively immune, are frequently affected, but usually by the lighter forms; the tertian is not infrequently seen in them. The Tamils, although largely occupied with hard field-labor, are the most resistant to the infection, and as a rule suffer from the quartan variety, when at all.

Simple Tertian.

This is characterized by febrile attacks which recur regularly every third day, with an intervening day of complete apyrexia (see chart No. 5). The average duration of the attack is from ten to twelve hours, only slightly longer than that of the quartan.

The same symptoms and the same course occur as in the quartan. It begins with a chill which is usually severe, but not so severe nor so prolonged as in the other ("frigida quartana" of the Latins). If the chill is absent, there are slight horripilation and subjective symptoms of cold—these are rarely lacking.

Grave symptoms are as a rule not present, even when the temperature is very high and the attack unduly prolonged. An exception must be made in the case of children and old persons; in the first we may have threatening nervous symptoms, as convulsions; in the latter we sometimes see a condition of profound weakness during or after the attack, and a state of delirium or subdelirium. Slight and transitory delirium may also be seen in adults.

The tertian may be primary; but very frequently a tertian fever

begins with an irregular or quotidian fever, becoming tertian later,

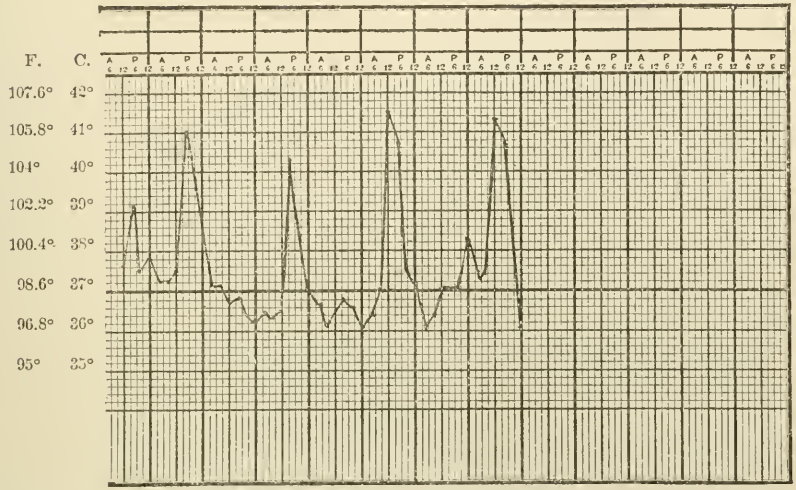


CHART No. 5.—Ordinary Tertian Fever.

either spontaneously or through the action of drugs. On the other hand, a simple tertian may become double.

If we follow accurately the temperature of a tertian patient, we shall quite frequently find slight elevations even on the days which should be apyretic, elevations which often entirely escape the observation of the patient. We do not often find a pure tertian—that is to say, one in which there is absolute freedom from fever—on the day intervening between two attacks. We might almost say that nearly all the tertians are potentially double tertians (see chart No. 6).

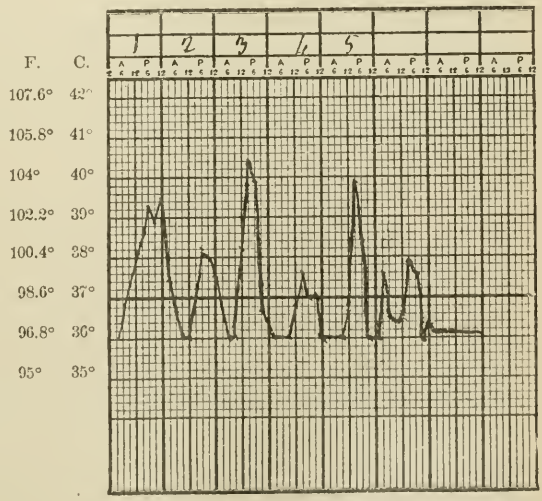


CHART No. 6.—Tertain Fever With Slight Elevation of Temperature on Intermediate Days Between the Regular Febrile Paroxysms.

The various attacks in a typical tertian may resemble each other as

to the hour of the invasion, the degree of the fever, and the duration. But it is a well-recognized fact that subsequent attacks may come a few hours earlier (tertian anteponens) or may, on the other hand, be somewhat delayed (tertian postponens). The first is the more frequent, and this is especially true in those cases which tend to become aggravated, although this cannot be stated as a general rule. A delay in the attacks is seen more seldom.

The tertian attack, like the quartan, is nearly always developed in the daytime, especially the first half of the day. When the attacks show regularity in anticipating their time, they may gradually recede towards the early morning hours, and finally become nocturnal, but this is not usual.

The attacks in the tertian and the double tertian may quite exceptionally occur at night.

Double Tertian.

This is a quotidian fever produced by two groups or generations of tertian parasites, which reach maturity and multiply (sporulate) at intervals of about one day.

The single attacks, which develop in the same manner as those of the pure tertian, may be alike in their duration and gravity, and begin every day at about the same hour. In this case there is a regular quotidian fever, whose tertian origin can be recognized only by an examination of the blood, or by the further clinical development. The cases, on the other hand, are not rare in which the paroxysms of the first and third days come at a stated hour, those of the second and fourth at another hour, and so on; and the correspondence of the attacks to alternate days may involve not only the hour of their appearance, but also the duration and the gravity of the fever. In such cases the diagnosis of double tertian can easily be made by observation of the temperature curve alone.

The existence of two generations or groups of parasites may, moreover, be demonstrated by the action of quinine, which by suppression of one group will cause the double tertian (quotidian) to become simple. This fact, long known to physicians, is explained by the statement of Golgi, that quinine when administered just before an attack destroys the generation of parasites producing that attack, but, unless given in very large doses, only slightly disturbs the group which is but half developed and which is to cause the attack of the following day.

A double tertian may, moreover, spontaneously become simple without the action of any remedy; this is not an infrequent occurrence in hospital patients under the effect of complete rest and good

food. In such cases, we may see for a while alternations of severe and light attacks, until the light attacks become completely exhausted and are mere elevations of temperature. Correspondingly one of the groups of parasites disappears from the blood.

On the other hand, a tertian may begin by being simple and later become double, as we see in chart No. 7. Not infrequently the simple tertian has slight elevations of temperature which go a little above 37° C. (98.6° F.) on the days between the attacks; at a given moment these elevations change into real attacks, and thus the tertian becomes double (see chart No. 7). In other cases the simple tertian

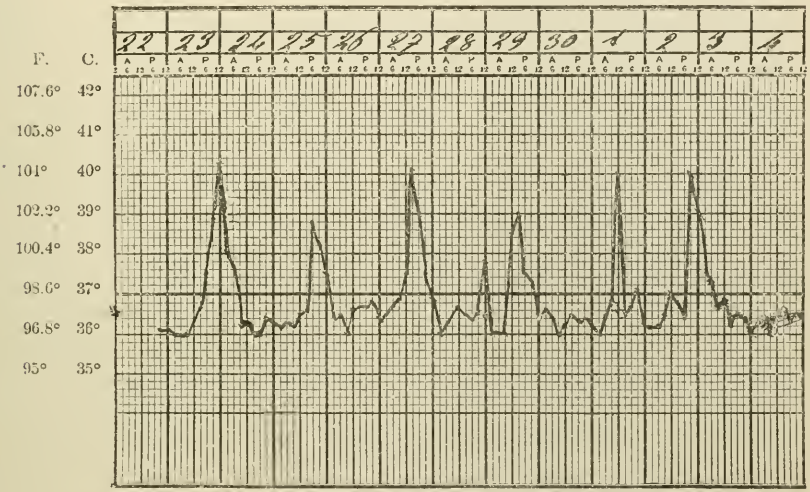


Chart No. 7.—Simple Anticipating Tertian Becoming Double.

is doubled suddenly without such a result having been indicated by anything in the course of the disease.

We have already noted that each succeeding attack of simple tertian may come earlier than the one before (anteponentes). When this occurs in a double tertian, the attacks may be incompletely separated, one beginning before the stage of sweating of the previous attack has quite finished (subintrant attacks). In order that this should occur, it is as a rule necessary that the attacks should not only anticipate their time, but that they should also be prolonged. This in our experience is very rare. For the most part, the attacks, even when they do anticipate, remain quite distinct. Not infrequently in the double tertian we find that when the attacks caused by a certain group of parasites on alternate days are in the habit of anticipating, the others also, due to another group of parasites, anticipate as well. This gives us the typical tracings of an anticipating

quotidian, whose tertian origin it is not possible to recognize from the curve alone (see Chart No. 8).

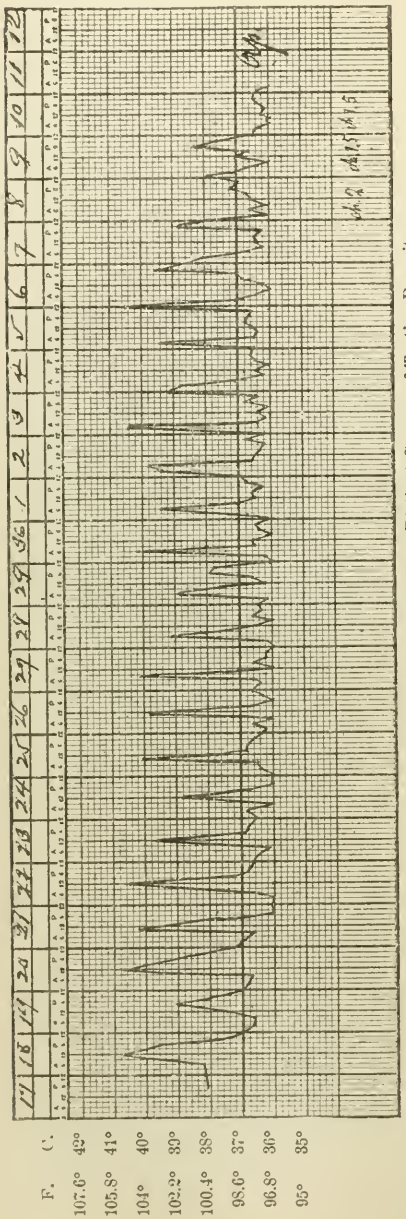


CHART No. 8.—Double Anticipating Tertian with Two Distinct Groups of Tertian Parasites.

In the cases in which a quotidian of tertian origin is followed for a considerable time without being modified by therapeutic measures, we are likely to see groups of severe and groups of milder attacks which alternate irregularly. It would seem as if there were oscillations in the pathogenic activity (perhaps in the activity of multiplication) of the parasites, or else in the capacity of the organism to resist this action or to rally from its effects. Such a problem as this is not easy of solution, especially when we consider that both the first and the second hypotheses contain a part of the truth. Even in the other forms of malaria—as, for instance, the estival—we often see groups of light attacks following severe ones, so that in some cases it seems to the physician that preparations are making for a spontaneous cure; then these are followed in turn by grave attacks, and a new aggravation of the infection occurs. Perhaps a partial explanation of these events may be found in what has been said in regard to immunity (see page 138).

Irregular and Subcontinuous Fevers of Tertian Origin.

These are less rare than the corresponding fevers of quartan origin, of which we have already spoken. It is not unusual to see irregular intermittent fevers of tertian origin generally in the first days

of the infection. Quite rare are the continuous fevers which from their general aspect remind one of a typhoid. As we shall see, this error in diagnosis occurs not infrequently in some forms of estival fever, and it was indeed a mistake often made before it became a matter of routine for the physician to make an examination of the blood.

Thayer and Hewetson speak of a case of "almost continuous" fever with quotidian exacerbations, which was at first supposed to be typhoid; after five days of treatment with baths, the fever became intermittent, and its tertian nature was recognized.

A similar case was observed by us in a young man in whom the fever lasted for about a week, with marked remissions at night; from the symptoms as a whole, experienced physicians judged the case to be one of typhoid. An examination of the blood, made only because the patient was known to have been staying in places where he might have taken malaria, demonstrated the tertian origin of the infection, which rapidly yielded to quinine.

The blood contains parasites in various stages of development, but because of the extreme rarity of these cases, parasitic researches, conducted methodically for a sufficient length of time to give reliable data, are lacking.

These subcontinuous fevers are of little practical importance, but it seems to us that the *prolonged attacks* of the ordinary tertian are worthy of attention. If we imagine a double tertian, in which two successive attacks merge into each other in such a way as to lose their individuality, we shall have the curve of a tertian with prolonged attacks.

In this way we may have attacks which will last thirty-six hours, separated by a brief and incomplete period of apyrexia of about six hours. The course of the fever resembles that of the estivoautumnal, from which, however, it is easily distinguished by an examination of the blood and by the further clinical course. In one case which we had under observation, the attack was prolonged without any notable oscillations for exactly thirty-six hours; in the blood we found parasites in various stages of development, grouped into two separate families, which matured about twenty-four hours apart. This form of tertian with prolonged attacks is very rare, and is apt to be irregular.

Quite exceptionally and by accident we had occasion to observe two attacks develop in one day (see Chart No. 9), but we have never seen a tertian continue for some time with two daily attacks. Since the ordinary attacks of tertian fever last on an average between ten and twelve hours, it is evident that it can occur only when the attacks are

short and light. It is well known that it is not rare to see two or even three febrile attacks in one day occurring with a certain amount of regularity in pyæmic fevers, such as puerperal infection, in biliary calculi, etc., and for this reason we think it not improbable that the majority of double quotidian fevers reported by physicians do not belong to the class of malarial infections, but are symptomatic of other diseases.

Examples of so-called *long-interval fevers* due to tertian parasites were first described by Bignami, and then by Vincenzi and others. In one case studied by Bignami the fever returned at intervals of from fourteen to sixteen days; in others it has been known to return

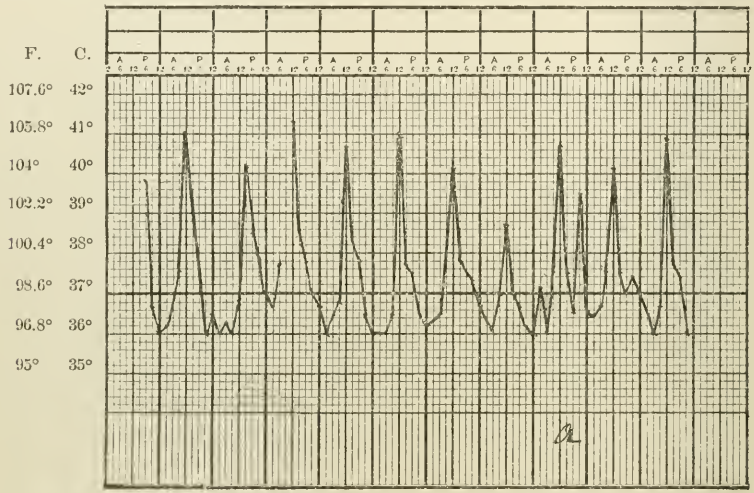


CHART No. 9.—Double Tertian. At *a* are seen two short paroxysms in one day.

at intervals of from seven to eight days. As these are cases of attenuated chronic infection, we shall treat of them later in the section to which they belong.

The Biological Cycle of the Tertian Parasite.

For a detailed study of the biology of the tertian parasites, we refer the reader to a previous section (page 61). In this connection we will content ourselves with stating the principal facts concerning the cycle of development of the parasite in relation to the development of the fever; that is to say, to the results obtained from an examination of the blood made in order to assist diagnosis and to explain the clinical facts.

If the blood is examined shortly after the febrile attack, we shall see in the red corpuscles young parasites (plasmodia of Marchiafava

and Celli) which are of small size, with a diameter of about one-fifth or one-quarter of the corpuscle itself. They have a diaphanous and whitish appearance, and their outline is not well marked in relation to the substance of the red corpuscle; yet they are easily recognized by their amœboid movements, which are much more active than those of the quartan bodies. The pseudopodia are slender and very long, frequently reaching to the periphery of the red corpuscle; they are sometimes so fine that we may not see their connection with the body of the parasite, unless they draw themselves in while we are watching them, other pseudopodia furrowing the substance of the blood corpuscles in other directions.

Bodies in a rather more advanced stage of development show fine granules of black pigment, which tend to accumulate towards the extremities of the pseudopodia, which are a trifle enlarged.

The red corpuscles containing these immature bodies may already be differentiated from the normal; they seem to have less tendency to shrivel, and appear, indeed, to be somewhat larger.

In the days of apyrexia (that is to say, at the beginning of the second day of the development of the parasite) the amœboid bodies are so much enlarged that they occupy from one-half to two-thirds of the corpuscle, are rich in pigment granules, and still endowed with amœboid movements, although to a less extent than in a younger stage. The pigment granules may be seen to move with activity and to change their places as though transported by a plasmatic current, even when the outline of the parasitic body cannot be seen to modify itself in any special way. The red corpuscles containing the parasites become pale, and are much larger than the others.

This increase in volume of the parasites and of the corpuscles containing them continues throughout the day of apyrexia. The red globule may become so pale that its outline can scarcely be perceived. The growth of the parasite rarely goes beyond four-fifths of the size of the corpuscle.

At this point occur the internal changes which precede segmentation, and segmentation itself, which occurs coincidently with the beginning of a new attack. According to Golgi, in very severe attacks segmentation begins a little before the commencement of the fever, and is protracted for about one or two hours after the fever has developed; in slight attacks, on the other hand, the segmentation appears to be completed before the attack begins. According to Golgi, segmentation occurs in several ways; he recognizes three forms:

1. The pigment collects at the centre of the adult pigmented body, the peripheral portion of this body forming a species of ring around a central pigmented disc; then the substance of this ring becomes

segmented into from fifteen to twenty little bodies, which are at first oval in form and then become globular, and which dispose themselves like a wreath around the residual hyaline substance which contains the pigment.

2. The pigment in the centre becomes reduced to a close mass, around which the parasitic body becomes entirely segmented, causing an accumulation of mulberry-like masses, which correspond to the form of fission described, even before Golgi, by Marchiafava and Celli.

3. In the adult pigmented bodies we note the formation of a vacuole, within which are one or two hyaline globules. Are these globules young parasites? Golgi expresses no opinion as to their significance, and, indeed, does not lay much stress upon them. More recent researches have quite justified this reserve.

The form of multiplication most frequently seen in tertian fever is that corresponding to the second classification of Golgi. The first variety has not been seen by other observers (Antolisei, for example). The third form, according to recent researches, must be considered as the result of degenerative processes.

Other variations in the mode of fission have been described. Thus Celli and Guarnieri found bodies undergoing fission, in which the pigment, instead of being massed at the centre, was disseminated through the daughter cells. The pigment may also be gathered into two or three groups, instead of only one. Not infrequently, instead of being in the centre, it is at some point near the periphery of the parasite. It is of more importance to know that in tertian fever there may be small, sporulating masses, formed by the accumulation of five or ten spores around one or several pigment granules, occupying little more than the half of the red corpuscles; these are very similar to the forms of fission which occur in the estival parasites, except that the spores are larger in the first. Bignami and Bastianelli have seen this form in a few anticipating tertians and irregular quotidians of tertian origin.

Not all the pigmented bodies in a tertian reach the stage of multiplication. Many are seen which become larger in size than the multiplying bodies, and two, three, and more times the size of the red blood corpuscles. Under the microscope we may not infrequently see them becoming filled with vacuoles, and then break up into hyaline spheres, which must be regarded as degenerative in their nature. Others become changed, while under observation, into flagelliform bodies, to the frequent presence of which, as forming a part of the cycle of development of the tertian parasite, Antolisei in especial has drawn attention. For more minute details upon the

morphology and significance of these bodies, we refer the reader to the earlier section of this work, in which the parasites are treated of at length.

In contradistinction to what occurs in the quartan, the adult parasites in the tertian *tend to accumulate in the internal organs*, as Bignami and Bastianelli demonstrated by systematic punctures of the spleen. Thus in the spleen a greater number of bodies in fission are found than in blood taken from the finger, and within the white corpuscles are found a large number of pigmented and hyaline spherules which have probably been derived from the breaking up of adult bodies, and blocks of pigment which have been set free after the disunion of the sporulating bodies.

A fact of importance to one who is conducting an examination for purposes of diagnosis is that bodies in fission cannot always be found in blood taken from the finger. This is explained by the statements made above, and has been noticed by all who have minutely followed the course of several successive attacks of mild tertian (Antolisei, Mannaberg, and others). On the other hand, we rarely find bodies in fission in the blood of the peripheral parts without a febrile attack following, as was noticed by Celli and Guarnieri; whilst it is not unusual to find fissions without fever in quartans which are becoming exhausted, a fact to which we called attention in a previous section. Not only may we find no fission forms in the blood, but, especially in the first attacks of a mild tertian, we may even find no parasites. This was observed by Antolisei during the early attacks of an experimental tertian, and we are able to confirm it for cases of spontaneous tertian. In the relapses, on the other hand, and also in a primary tertian after several attacks, characteristic parasites are always found in the blood, even when the fever is of a mild type.

When apyrexia has been obtained by means of quinine, and even when the temperature has fallen spontaneously, we have several times found in the blood the large bodies undergoing a breaking-up process, to which we referred above; and these may persist for several days as the only representatives of an infection which is becoming exhausted (Bastianelli and Bignami). With these we may find pigmented white blood corpuscles.

As having a diagnostic significance we would call attention to the presence of pigmented spherules of varying sizes, which are almost always found free in the blood of a tertian patient. They are of importance in diagnosis, because sometimes they exist alone, the most careful examination failing to reveal any endoglobular parasitic bodies. These spherules are composed of a hyaline substance with small granules of freely moving pigment, and possess so characteristic an

appearance to the expert eye that they quite suffice to a diagnosis of malarial tertian. For the greater part, they are merely parasites which have escaped from the red corpuscles. Not infrequently we may, during a microscopic examination, witness their exit; the parasite first forms a hernia from the globule, which suddenly becomes decolorized, and the pigmented hyaline body becomes free and takes on a spherical form. Sometimes at the moment of this escape it seems almost as if the red corpuscles were bursting. In some cases only a part of the endoglobular pigmented body escapes, separating itself by tearing away from the portion which remains within the corpuscle. There is reason to believe that the same process can occur in the circulating blood, as we have noted in a previous section.

As a result of what has been demonstrated, Golgi's law, that the multiplication of a group of parasites coincides with the beginning of a febrile attack, is seen to apply to the tertian type of fever. Bodies in complete fission may be found two or three hours before an attack, but not five or six hours, as in the case of quartan fever. In specimens properly stained for a study of the structure of these bodies, the first stages of division of the nucleus into four, six, and more parts can be seen six hours before the attack.

In double tertian, two groups of parasites are found in the blood, which mature at intervals of about twenty-four hours; so that if an examination is made, say, towards the beginning of an attack, we shall find bodies undergoing or having undergone fission, and endoglobular parasites which have reached about the middle stage of their development, and which will mature, divide, and cause fever on the following day. In anticipating tertians the probable cause of the tendency to anticipate is a more rapid development of the parasites. As to the anticipating tertians which become aggravated in type, the explanation of Feletti seems to us to be valid; this is that in every tertian we find bodies in fission several hours before the appearance of the fever, which continue into the beginning of the attack; the greater the number of parasites the more quickly will the matters necessary to the production of fever accumulate in the blood—hence the anticipating type.

Clinical Course.

The clinical course of a tertian may be gathered in part from what has already been said. As we know, the disease often begins with an irregular or quotidian fever, and it is only later or by the action of quinine that it develops into a tertian, properly so called. According to our experience, the double tertian is more frequent than the single. Such is also the experience of Thayer and Hewetson, who

(in Baltimore) observed one hundred and fifty-one cases of pure tertian, and one hundred and eighty-eight of the double. Physicians in the tropics make the same statement. We have already called attention to the relative frequency of the triple quartan in primary quartan infections. We lay particular stress upon that of the tertian, because the fact is probably of some theoretical value, and may be satisfactorily explained by taking into account the views as to the manner of absorbing the infection from the surroundings (see the section on Etiology, page 109).

Left to itself, the fever tends to a spontaneous cure, after a series of more or less numerous attacks. Such a cure sometimes comes after several attacks of gradually decreasing severity, sometimes after an intense and prolonged attack. Spontaneous recovery is often seen in hospitals; the patients, taken from hard labor in the country, put to bed, and given abundant nourishment, have one, two, or three attacks, and then recover. As a rule, however, after a variable interval of apyrexia, relapses occur, which are apt to be milder than the primary attack and which also tend to a spontaneous cure. By a well-directed administration of quinine relapses may be delayed, attenuated, and even prevented. Although, as we have already stated, in the greater number of patients who naturally take quinine, we may successively find the various febrile types to which the tertian can give rise, yet there is a certain tendency in the simple tertian to relapse as such, and the same is true of the double tertian. Even the older physicians observed that the ordinary tertian was less obstinate in its relapses than the quartan, and as a rule lasted a shorter time.

In reference to the gravity of the attacks and the possible sequelæ, we must not suppose that the tertian infection is a more serious type than the quartan, simply because it is produced by a parasite which has a more rapid development. Everything tends to support the opposite view. We have seen that the condition of anæmia which follows the tertian can be overcome with a certain amount of rapidity, especially in comparison with that which follows the estival type. From our experience we are unable to assert that the most serious of all the sequelæ—malarial cachexia—is caused by the tertian alone; all the cachectics seen by us had suffered from various kinds of malaria, among them the estival. The tertian certainly, in its relapses, may take on the appearance of a chronic malaria (earthy complexion, enlarged spleen), the patient nevertheless still having sufficient strength and capacity to pursue farm work. Even in the beginning of a tertian we do not find pernicious attacks. We may, however, observe during the attack phenomena of a certain gravity, especially

when the temperature is very high and unduly prolonged, or when the patients are already enfeebled. Thus in aged patients, those who were weak and suffering from arteriosclerosis, we have often noticed great exhaustion following a few attacks of tertian fever. But even in such cases, in which the gravity is due to special individual conditions, pernicious symptoms, properly so called, are absent. Neither do we have them in the irregular and subcontinuous fevers, which for several days, in the absence of an examination of the blood, might cause us to suspect typhoid.

Mixed infections of tertian with other varieties of malaria have often been observed by us. Thus we have seen cases of tertian and quartan infection which as a rule were manifested by an irregular fever. Variations of this type have been studied by Bastianelli and Bignami. The most important of the mixed infections is the *tertian and estival.* In the summer it is rather usual to see patients who give the symptoms and the fever course of an estival tertian, and in whose blood a few tertian parasites are found, in addition to the estival parasites. As a rule, the first do not in the least modify the course of the predominant infection; they are seen only in the first attacks and then disappear, but it is important to note that they may return in the relapses, and even in very late relapses, giving an ordinary single or a double tertian. As a consequence, in cases in which we observe a patient, even after several relapses of an estival type, begin to have relapses of an ordinary tertian variety without there having been any exposure to further infection, we conclude that there has been a long period of latency for tertian parasites which, after gradual attenuation of the estival parasites, gradually take the ascendant. From our standpoint, this would explain the fact that a patient entering the hospital in the autumn with an estivoautumnal fever, and remaining there, may after a few months, say, in the late winter, have a relapse with tertian parasites. These cases, as is well known, have given rise to long discussions upon the possibility of the transformation of one form of parasite into another—a possibility which may now be altogether excluded.

For *treatment* the reader is referred to the general section on Treatment (page 446 *et seq.*). We would here merely call attention to the fact that it is in this form of malaria, and because of its tendency to recover spontaneously, that many observers have obtained such remarkable results from the various substances which have come in vogue of late years, such as methyl blue and phenocoll, substances which, however, do not possess the specific action of quinine.

For a discussion of the *pathological anatomy* of both tertian and quartan, see the general section on that subject (page 227 *et seq.*).

Estivoautumnal Infection.

History.

In addition to the tertian and quartan fevers which in the spring of the year so dominate all other types that, *a potiori*, the name of *vernal fever* has been given to them, malarial infection may cause another variety, which in temperate climates occurs in the summer and autumn and predominates over the first.

All physicians living in malarial regions clinically recognize the estivoautumnal fevers, and have observed the gravity of the attacks, their long duration, subcontinuous nature, etc. Their tendency to become malignant was noticed even in the time of Hippocrates, who in one of the books "Epidemiorum" wrote: "Febres tertianas in æstate ortas aliquando malignas fieri; et in febres continuas, acutas mutari; et proinde cavere oportet; id est timere et curare caute."

These fevers, which are unknown in cold climates, begin in temperate regions (where all the clinical varieties of malarial infection are manifested) towards the end of June, or more frequently early in July, after the occurrence of extreme heat. If a period of heat is succeeded by decided cooling of the atmosphere, especially when the latter is preceded by rain, then we may expect (in the neighborhood of Rome) that for several days in succession a number of field laborers will come down with the disease. With some diversity in the number of patients affected, the fever is prolonged through the summer and continues into the autumn, undergoing some exacerbations after the first rains, and lasting a variable time, according to the conditions of the season. In warm and moist autumns, unmodified by the north winds, the infection persists up to December, and even to the end of that month, ceasing abruptly with the advent of continued cold weather. From this period primary estivoautumnal fevers come to an end; but throughout the winter we may see relapses in those who became infected in the summer and autumn. We may say that in the winter there is an arrest in the production of malaria, which, however, becomes active again in the spring, giving rise to epidemics of a mild type, especially the tertian. We prefer to use the name of estivoautumnal fever for this type, as being the one accepted by the greater number of writers, and the most convenient for the present, at least in temperate climates; but we are aware that other names have been suggested or inferred from the morphology of the parasite producing it, or from some clinical peculiarity.

In reading the clinical descriptions of this fever given by the

various writers before the discovery of the malarial parasite, we are surprised to find how great a confusion exists, due to contradictory descriptions, to the various significations given to the same term, to the including of fevers which were certainly not malarial, etc.; and we are inclined to agree with those who, like Sternberg and Dutrouleau, cry: "This is chaos!"

Some of the older writers appear to have been more accurate than some of the more recent ones who wrote before the discovery of the parasite. Thus Sydenham divided the fevers into winter and autumnal; the first, he said, were not dangerous, although of longer duration; the second had an entirely different course, being unrecognizable in the first attacks, resistant to treatment, of graver nature, and leaving more serious results, as dropsy. According to Sydenham, the distinction was a fundamental and essential one, the autumnal fever being entirely different from the other: "Non dubito quin febres istæ tota sua natura, sive essentialiter, distinguantur."

The best part of our clinical knowledge of these fevers dates from the work by Torti, the great value of which is recognized by all, not only because of its happy descriptions of the various febrile types, but because the author strenuously insisted that the disease could be cured by cinchona bark.

Torti divided the intermittent fevers according to their type into quotidian, tertian, and quartan. When distinctly intermittent he called them *discrete; subintrant* when an uncompleted attack ran into the succeeding one. He distinguished the tertian fevers as benign and malignant; the pernicious he divided into the *solitary* (solitariæ) and *complicated* (comitatæ), "videlicet," as he writes, "in eam, quæ ex prava natura sua ad acutam, malignam et pernicialem vergit, et in eam, quæ talis repente fit ob adjunctum ferale aliquod et peculiare symptoma, morbo ipso, quem mentitur, et febre, quam comitatur, deterius." The complicated pernicious fevers he divided into *choleraic, subcruent, cardiac, diaphoretic, syncopal, algid, and lethargic.* Simple pernicious fevers ("febres subcontinuæ perniciosæ seu malignantes") were those in which the fever became continuous, especially by the overlapping of attacks, and which were accompanied by grave symptoms of various kinds.

Nearly all other writers have adopted Torti's classification and fundamental theories, without adding facts and observations of much importance; so much was this the case that we are obliged to come down to the last twenty or thirty years to find anything worthy of attention.

Baccelli, since 1866, has devoted himself to the study of malarial fevers. To refer only to that part of his work which relates to the

type of fevers under discussion, he gave special attention to subcontinuous fevers and to the differential diagnosis between these and subintrant fevers, and intermittent fevers complicated by some other disease characterized by a continuous fever. "Subcontinuous fevers sometimes begin as intermittent fever, and again are subcontinuous from the first. In the first instance we see the attacks which at the beginning are distinct and autonomous, so to speak, become more and more frequent, until finally their paroxysmal type is lost in the appearance of continuity; in the second case, the occurrence of attacks is manifested only by an increase and diminution of temperature at short, very short intervals." Subcontinuous fevers are pernicious because of their type; that is to say, "by the numerical increase of the attacks in a given time." The complicated pernicious fevers of Torti are due to the slight resistance of the organ in which the chief symptoms are shown. As to the clinical form, the difference between the complicated and the subcontinuous consists in the fact that while the first has some chief characteristic symptom, such as coma, the second is characterized by some distinct and complete morbid form, such as the pneumonic or typhoid, with which two Baccelli chiefly concerned himself. After the discovery of the parasite, Baccelli observed that in subcontinuous fevers several generations of parasites coexisted, which impinged upon each other, even as the febrile attacks which they occasioned.

Colin, who pursued his investigations in Rome, clearly distinguished the group of estival fevers, whose intermittent nature, however, he failed to recognize in the majority of his cases, and which he therefore called *remittent fevers*. To these remittent fevers, which were almost always initial, succeeded the distinct periodical ones. As to the pernicious fevers, he preserved the division of Torti into complicated and solitary (febres comitatæ et solitariæ). He recalled the fact that Torti and then Puccinotti observed that solitary or subcontinuous pernicious fevers are very frequently accompanied by symptoms belonging to the complicated fevers, and hence concluded that the name subcontinuous remittent was better than solitary, because, while it expressed the fact of the continuity of the fever, it did not exclude the possibility of the occurrence of the grave symptoms which belong to the complicated forms. The pernicious fevers observed by Colin are the comatose, delirious, convulsive, algid, choleraic, syncopal, and subcontinuous. Of the last he distinguished two forms: (1) Subcontinuous estival (ataxic, typhoid, remittent typhoid). This is developed during periods of intense heat; it may follow attacks of periodical fever, but occurs especially during a simple remittent fever. The duration of the latter is pro-

longed, the symptoms are aggravated, and the patient falls into a typhoid condition. In some cases there are found at the autopsy the most characteristic lesions of typhoid fever; in others the alterations of pernicious fevers. The author does not interpret the first as mixed infection, but holds that the malarial infection has been transformed into a typhoid infection (?). (2) Subcontinuous autumnal fever; this is the subcontinuous of cachectics, and occurs in individuals who have or have had intermittent fevers. While the fever becomes continuous, grave symptoms appear—epistaxis, nocturnal delirium, muscular subsultus, hypostatic pneumonia, bedsores, and paralysis. At the autopsy no typhoid lesions are found. Of this form of subcontinuous fever, Colin reports only one case. He attributes great importance to the influence of quotidian and remittent fevers in the production of pernicious fevers. In Algeria, pernicious fevers are most frequently found in patients suffering from remittent fever; next, from quotidian; and lastly, from tertian. As to the transformation of the intermittent fevers from one type to another, the writer considers this to be quite exceptional. In patients, he says, who remain a long time in the hospital, the attacks as a rule continue with a decided rhythm; only after their discharge from the hospital may the type sometimes vary. If the transformation occur in the hospital under the eye of the physician, it usually takes place at the onset, a quotidian changing into a tertian, and *vice versa*. He does not, with Griesinger, admit the progressive change by which the passing of one type into the other would be merely the consequence of a series of anticipated or delayed attacks.

Sternberg includes all malarial fevers which are not distinctly intermittent in the chapter upon "remittent fevers," of which the greater number in the United States occur in August; of these fevers he recognized various forms: (1) A simple malarial remittent; this is a paroxysmal fever, differing from the intermittent by a more prolonged attack which is not followed by complete apyrexia, and by a cold stage which is ill defined or absent. In grave cases symptoms of gastric irritability and biliary vomit are frequent. He reports cases of quotidian remittent fever, double tertian, etc. (2) Ardent malarial fever. This is rather continuous than remittent in type; it dominates in tropical regions, but is also prevalent during the hot season in subtropical climates and in the southern temperate regions. (3) Adynamic remittent or subcontinuous estival fever. This is a type in which, instead of yielding to the specific treatment in the usual time or terminating in a simple intermittent fever, the disease is protracted with adynamic symptoms accompanied by high or by slight fever of an irregular character. (4) Pernicious remittent fever includes a

great number of pernicious types with a remittent fever, and the symptoms which we have in the complicated fever of Torti. (5) Complicated remittent fever, which may have cerebral, gastric, or enteric complications.

Kelsch and Kiener divided the intermittent fevers as follows:

Solitary fevers (febres solitariæ) { Simple fevers. / Gastricobilious fevers / Typhoid adynamic fevers.

Complicated fevers (febres comitatæ) { Group characterized by cerebral symptoms. / Group characterized by gastrointestinal symptoms. / Group characterized by hæmatolysis.

Solitary Fevers.—(1) The simple malarial fevers are intermittent or remittent, and correspond to a milder type of intoxication. The remittent fevers are composed of febrile periods of variable length, separated by more or less incomplete intermissions, resulting from one prolonged attack in which the temperature may remain above 40° C. (104° F.) for thirty-six or forty-eight hours or more, or from several subintrant attacks. (2) The gastricobilious fevers predominate during the recrudescent epidemic which annually occurs with the advent of hot weather, they may be of the tertian, quotidian, or remittent type, but the last is the most usual. Among the chief symptoms are those due to the disturbance of the gastrointestinal and hepatic functions; that is to say, epigastric pain, bilious vomiting, diarrhœa, pain in the hepatic region, icteric or subicteric staining of the skin, frequent albuminuria, highly colored urine, especially from urobilin, epistaxis, headache, etc. The writers insist that polycholia exists in this form of fever, and thus explain the icterus. (3) The pernicious solitary fevers, of a typhoid or adynamic type, correspond to the malignant subcontinuous fevers of Torti, and are accompanied by grave symptoms similar to those of the complicated variety; that is to say, stupor, delirium, cardiac debility, and termination in coma. The adynamic form is distinguished by its long duration, the profound anæmia, the jaundice, the slight elevation of temperature, or even hypothermia. This variety corresponds to the subcontinuous autumnal fever of Colin.

Complicated Fevers.—Of these the cerebral are the most frequent; they come next after the pernicious solitary fevers, to which they are often intimately joined. Under the name of algid pernicious fevers, these writers include the cardiac, the choleraic, the dysenteric, the diaphoretic, and the syncopal. The observations of Kelsch and Kiener were made in Algeria.

Schellong observed in the intermittent fevers a domination of the quotidian type, a tendency on the part of the attacks to anticipate,

obscure periods of apyrexia, and frequent absence of the chills. The atypical, continuous, or remittent fevers as a rule last about a week, and are most frequently observed at the acme of the malarial epidemic. The symptoms are mild in some cases, grave in some, and in others of the most pernicious type with a fatal issue; in the last-named the gastroenteric symptoms described above are frequent. Europeans are the most apt to be attacked by this variety of fever, upon which quinine has but little influence. In addition to these febrile types, Schellong described *hæmaturic bilious fevers*, a very dangerous form, characterized by acute jaundice and hæmoglobinuria; and *comatose malaria*, the gravest pernicious form, an expression of the influence of the malarial virus upon the nervous centres. Under this name Schellong includes other varieties of pernicious fevers, as the *eclamptic*, the *tetanic*, the *delirious*, etc.

This ends the list of some of the writers who concerned themselves with the grave malarial infections previous to the discovery of the parasite, or after its discovery but without making an examination of the blood. Even as we may be sure of the malarial origin of the intermittent fevers and also of many of the subcontinuous and remittent ones, so, on the other hand, we may be permitted to doubt that some of the fevers of this last group described by many of the authorities referred to were in reality of malarial origin. The doubt is based upon the clinical description, the anatomicopathological findings, or the absolute non-influence upon them of the specific remedy for malaria. In a detailed study of the estivoautumnal fevers, we shall see how in malarial seasons and climates infective fevers occur whose etiology is not yet known, and which, without an examination of the blood, might even at the present day be confounded with diseases of malarial origin.

Laveran, the discoverer of the malarial parasite, was naturally the first to confirm his observations by an examination of the blood. He divided malarial fevers into *intermittent* and *continuous*. As to the frequency of the various febrile types and the season at which they were most frequently observed, he noted that the quotidian fever is the most frequent manifestation of malaria in Algeria; that the intermittent tertian and quartan are essentially relapsing fevers; that the continuous fevers are observed only in the hot season; that at this time the quotidian fevers are the most common of the intermittent variety; that during the cold weather continuous fevers disappear and the tertian increases. As to continuous malarial fevers, the principal facts of which Laveran speaks are the following: The greater number are observed in the months of July, August, September, and October (in Constantine, Algeria). The causes for the con-

tinuity are (*a*) the external heat, (*b*) the marked reaction produced in vigorous persons, especially by a first attack of malaria (continuous fever seldom attacks natives or old fever patients, but chiefly newcomers to the malarial districts—Annesly, Griesinger, Colin), and (*c*) the intensity of the infection. Laveran holds that continuous malarial fever is derived from quotidian, by prolongation and overlapping of the attacks, without chills. The symptoms are onset without chill, a constant headache which is usually frontal, lumbar pains, great heat, a tongue either saburral or red and dry at the tip, great thirst, complete anorexia, sometimes diarrhœa, but more often constipation, frequently splenic pain which is spontaneous or caused by pressure, swelling of the spleen so slight as to be rarely appreciable to palpation, frequent epistaxis, nervous symptoms similar to those of typhoid fever, and prostration and apathy, or agitation and anxiousness. In some patients there are symptoms of pulmonary congestion or bronchitis; in others there is vomiting of biliary matter with more or less marked icterus. If these fevers can be cured by quinine, they are rarely prolonged more than four or five days. The temperature curve is atypical. At the crisis there are oscillations of temperature of greater or less extent; the night temperature is as a rule higher than that of the morning, but there are many exceptions. The parasites found in the continuous fevers, as in the quotidian fevers at their first invasion, are the *No. 2 bodies* or *spherical bodies*, and sometimes we find only the No. 2 bodies of the smallest size. Laveran did not attempt a classification of the pernicious fevers; there are none properly so called which form a clinical variety, but they are only complicating accidents of the ordinary malarial fevers. From this *résumé* of the clinical description of the continued fevers given by Laveran, we see that among them are not to be found the varieties of type named by other authorities, as, for instance, Colin; nor does he include among malarial fevers those fevers of long duration which resist the action of quinine, of which the other writers speak. An examination of the blood enables him to avoid falling into such errors of diagnosis.

The more recent researches made in regard to estivoautumnal malarial infection have led not only to a clearer appreciation of the febrile types which spring from them, but also to a deeper study of the parasite producing them, and the relation between its biology and the development of the febrile type. This is a study of the utmost importance, when we consider that to this group of fevers belong the gravest manifestations of malarial infection, including pernicious fevers.

Already at the time of Golgi's researches into the species of

parasites of the quartan and tertian fevers, no one interested in the subject could fail to notice the great difference between the parasite described by Golgi at Pavia, where the tertian and quartan fevers predominate, and those described by Marchiafava and Celli on the Roman Campagna and the Pontine Marshes, where, in addition to these two varieties, there predominate in the summer and autumn other fevers of a different type and graver form, among them the pernicious fevers. These investigators described in the Roman fevers the presence in the blood of endoglobular parasites, which appeared as small amœbæ, pigmented or non-pigmented; and in the case of lethal pernicious fever the presence in the capillaries of the organs, especially the brain and spleen, of small parasites in a more advanced stage of development and even of fission forms.

But it was in the summer of 1889 that Marchiafava and Celli first clearly differentiated the parasite of estivoautumnal fevers, and described its morphology and biology, showing the relation between its various phases and the course of the fever. These writers established the group of the estivoautumnal fevers on the basis of their endemic, clinical, and parasitical characters. As to the parasites producing these fevers, they recognized that they were so distinct from those of the simple tertian and the quartan that it is possible to make the differential diagnosis at the first microscopical examination. These parasites appear at first as small, non-pigmented, endoglobular amœbæ, in size from one-eighth to one-fifth that of the red corpuscle, sometimes endowed with active amœboid movements, and when at rest taking on the well-known annular shape. As the febrile attack progresses, the parasites show fine pigment granules, and their movements become less active. At the end of the attack the parasites, which have now become round and are always relatively smaller than those of the other types of fever, contain a mass of pigment which is usually centrally situated; at this time we also find many of the parasites within shrunken red corpuscles, which are deformed and have irregular outlines (brassy bodies); these parasites soon disappear, and with a fresh attack we see again the small, non-pigmented, endoglobular amœbæ. Another fact characteristic of this species of parasite attracted the attention of these authors, namely, that sporulation was not seen, in the great majority of cases, in blood taken from the finger. The sporulation of these parasites occurs in some of the internal viscera, as was found by examining the organs of persons who had died from pernicious fever, and comparing the results with those obtained from the examination of blood taken from the skin shortly before death, and of the blood of the spleen during life. Finally, they found that when the infection had

already lasted some time, especially in the autumn, crescent bodies were found in the blood, whose endoglobular development they were able to follow in its every stage.

The accuracy of these fundamental data in regard to the parasites of estivoautumnal fever, first established by Marchiafava and Celli, has since been recognized by all other investigators up to the present day.

Shortly after the report published by Marchiafava and Celli, Canalis published a report, in which, taking no account of the work of the above-mentioned authors, he held that the variety of parasite producing grave fevers was the crescent body, which completes its cycle of existence up to sporulation, whence the formation of the small amœba in relation to the febrile attack. In a succeeding work he further asserted that the parasite completes two cycles—a rapid one made by the small amœba, the description of which tallies in all essentials with that given by Marchiafava and Celli; and a slow cycle made by the crescent body, whose endoglobular development he confirms, and he describes the sporulation. To this variety of parasite are related the irregular and continuous fevers, the pernicious fevers, and even the malarial cachexia and the long-interval fevers which Golgi also held to be due to the crescent bodies.

Antolisei and Angelini recognized that the estivoautumnal fevers are produced by the crescent variety of parasite. In their opinion the crescent bodies of Laveran are the adult form of the amœba described by Marchiafava and Celli, from which would occur fission in the mode described by Canalis. They admit, however, that sporulation may occur prematurely in the small endoglobular amœbæ without their passing through the crescent phase, usually after pigmentation, but sometimes before the formation of pigment, as Marchiafava and Celli had already observed. These authors said that "this variety of parasite gives rise to so variable a febrile course that from none of our thermographic charts can we deduce how much time it takes the parasite to go from the amœba phase to sporulation. The course of the fever has not been observed to be rhythmic."

Grassi and Feletti, from a series of researches upon the malarial parasite of man and similar parasites in the blood of birds, believe that they are in a position to affirm that the crescent bodies represent a special species of parasite quite distinct from the estivoautumnal amœba. As has already been mentioned, they distinguish two kinds of malarial parasites in man: the Hæmamœba and the Laverania. In the first genus are included the H. malariæ, which produces the quartan fever; the H. vivax, cause of the simple tertian; and the H. præcox and H. immaculata, corresponding to the estivoautumnal par-

asite of Marchiafava and Celli, to which are due the grave estivo-autumnal fevers. The genus Laverania is represented by the crescent form, in which they admit sporulation, the cause of quotidian and subcontinuous fevers, and the long-interval fevers.

Bastianelli and Bignami, as a result of their observations, hold that the amœba præcox and the crescent body or Laverania of Grassi and Feletti are two forms of the same parasite. The crescent forms appear after several febrile attacks, first in the blood of the spleen, then on the eighth or ninth day in that of the finger. These bodies, which they never saw in sporulation, persist after the administration of quinine, during the periods of apyrexia, and in the relapses, during which, however, the fever-producing amœbæ return and go through their cycle of existence. Considering their mode of origin, the absence of sporulation, the analogy of the crescent bodies with the large pigmented tertian bodies which never sporulate but may become flagellate, these authors incline to believe that the bodies in the crescent stage represent in man the sterile form of the parasite in this group of fevers.

Sakharoff describes a parasite of the irregular fevers which corresponds to the estivoautumnal variety of Marchiafava and Celli, except that Sakharoff frequently found sporulating forms in the blood of the peripheral vessels. As to the crescent bodies, he agrees with Grassi and Feletti that they are a special variety of parasite.

Marchiafava and Bignami returned to a study of the clinical course of the estivoautumnal fevers and the parasites which produce them. They conducted their investigations with the view of ascertaining whether the irregularities considered by so many to be the characteristic feature of these fevers were not apparent irregularities only, and whether it would not be possible to demonstrate the clinical type or types of these fevers so as to have a starting-point for the understanding of the complex forms; and, in this event, to ascertain whether there were a relation between the parasitic data and the course of the fever. As a result of their observations they distinguish two fundamental types of estivoautumnal fevers, the quotidian and the tertian. In the former the attacks, which occur daily, are of variable length—now long, now short, now prolonged so as to impinge into the next attack, and thus cause a subcontinuous type which is more frequent during the first invasion of a fever than in the relapses. In the estivoautumnal tertian or malignant fever, so called to distinguish it from the spring or benign tertian, the attacks come on alternate days, but the single attacks are prolonged for twenty-four, thirty-six, or forty hours, so that the period of apyrexia is brief, very brief, and often obscured by the serious discomfort which

persists. In the temperature curves of the single attacks we find characteristic modifications of the temperature. The estivoautumnal tertian is a tertian with prolonged attacks. But its course may become modified, like that of the quotidian, by prolongation, anticipation, delay, or reduplication of the attacks, modifications in the curve of the attacks causing the frequent subcontinuous type, as in the quotidian fevers, to be manifested in the attacks of a primary invasion. As to the parasites producing these fevers, Marchiafava and Bignami, while they confirmed the observations previously made by themselves and other investigators, found a few morphological and biological differences between those seen in the estivoautumnal quotidian fevers and those in the tertian—differences which refer to the duration of the cycle of development to the pigmentation, the size, etc.; and in regard to which they incline to the view that in the estivoautumnal parasites there are two closely related varieties, that of the tertian and that of the quotidian.

Mannaberg divides the malarial fevers into two groups, corresponding to those of Marchiafava and Celli; that is to say, into (*a*) fevers produced by the malarial parasites with sporulation, but without the formation of syzygia (these include the tertian and quartan fevers), and (*b*) fevers produced by the parasites with the formation of syzygia; that is to say, with the formation of crescent bodies. This second group corresponds to our estivoautumnal fevers, in which Mannaberg also distinguished the quotidian and the malignant tertian types. Of the estivoautumnal parasites he, as we have already said, distinguished three varieties: (*a*) the pigmented parasite of the quotidian; (*b*) the non-pigmented parasite of the quotidian, corresponding to both the hæmamœba præcox and the hæmamœba immaculata of Grassi and Feletti; and (*c*) the parasite of the malignant tertian. We have already alluded to the explanation of the crescent bodies given by Mannaberg.

Golgi holds that the division of the estivoautumnal parasites into the parasite of the quotidian and that of the tertian malignant is not founded upon convincing demonstrations; that this parasite, whose cycle of existence is most irregular, or at least not yet well determined, and always longer than is held by Marchiafava and Bignami, develops entirely within the internal organs, also within the white cells and the endothelial cells; that therefore the parasitic bodies which are found in the circulating blood are there accidentally only—they are not an essential sign, although almost constant in these infections, and although they are in large measure degenerating bodies, they may accumulate and even multiply in the internal organs; finally, that the estivoautumnal fevers connected with these parasites,

whose chief seat is the internal organs, have a multiform type, so frequently irregular that it is as yet impossible to differentiate them upon a parasitico-biological basis, as has been done in the case of the classical intermittent fevers.

Feletti, who studied these fevers in Catania (Sicily), in the group of estivoautumnal fevers distinguishes (*a*) those due to the *amœba præcox*, which are seen exclusively in the summer, are characterized by their gravity, and are either subcontinuous or of the tertian type described by Marchiafava and Bignami, the type which he regards as a quotidian, with attacks now delayed and again anticipating; the cycle of existence of the parasite lasts for twenty-four hours; (*b*) fevers due to the *Laverania malariæ*, which are irregular in the duration of the single attacks and the intervals of apyrexia separating the attacks or the groups of quotidian attacks. These fevers present no pernicious symptoms, and the developmental cycle of the parasite producing them is very variable—a fact which corresponds to the course of the fever.

Thayer and Hewetson recognize the group of estivoautumnal fevers with the two intermittent quotidian and tertian types, as described by Marchiafava and Bignami. In a few cases an examination of the blood made during the course of the fever suggested the idea that the cycle of existence of the parasites was from twenty-four to forty-eight hours. But they realized that there are many intermediate intermittent types of fever with long paroxysms and irregular intervals, and even some continuous fevers, in which it is not possible to find any fundamental type, but which are all due to the same estivoautumnal parasite. Indeed, the writers were unable to distinguish any morphological or biological difference in the parasites associated in the various febrile types, and hence they do not agree with the division of the species into the parasite of the quotidian and that of the malignant tertian. As to the pathogenesis of the irregularities in the febrile manifestations, Hewetson and Thayer incline to attribute them to an irregularity in duration of the life-cycle of the estivoautumnal parasite, to the greater duration of the period of sporulation for single groups of parasites, and, finally, to the fact that very frequently parasites are present in every stage of development, and sporulation occurs almost continuously.

Welch and Thayer (1897) have published a most detailed work upon malaria; the first part treats of the parasites, the second of the clinical aspect, and in this they report practically the same results as those obtained with Hewetson, which we have already quoted. To the estivoautumnal parasite Welch gives the name *hæmatozoon falciparum*.

Osler, who had previously published works upon malaria, in a recent article upon the subject distinguishes in the estivoautumnal fevers three types: (*a*) The intermittent irregular fevers, with long paroxysms, which have a tendency to approximate and run into each other, thus giving continuity. (*b*) The continuous or remittent fevers, which sometimes begin with a severe chill, and are often preceded by headache, nausea, vomiting, and pain in the lumbar region and the limbs; gastric symptoms with icterus occasionally appear; the fever continues with remissions for a period of from one to two weeks; it varies in its degree of gravity, but in the more severe forms we have pernicious symptoms; in these continued fevers are included those called gastric remittent, typhoid remittent, and bilious remittent. (*c*) The pernicious fevers.

It will be seen from this recapitulation of the chief researches made in regard to the estivoautumnal fevers after the first reports published by Marchiafava and Celli, that while all writers are agreed that this type, including the pernicious form, is due to the parasite described by these investigators, there is a difference of opinion as to the clinical course of the disease. Some merely consider it to be irregular; others, while admitting that it is not distinctly periodical and that the attacks by their prolongation and by subintrance tend to lose their identity (whence the subcontinuity), describe one or two febrile types, the quotidian and the tertian, to which are related the changes in the parasitic cycle or cycles. However diverse the opinion held by the various authorities concerning the clinical course of these fevers and the biology of the parasites, it is very certain that since the discovery of the latter, with the assistance given by the examination of the blood, recent investigations into the clinical aspect of the grave malarial fevers, while they have more clearly described the various symptoms and their pathogenesis, have led to an elimination of the febrile diseases which used erroneously to be considered as malaria, but which differed from it in their course, their resistance to quinine, etc. They are still considered malarial in certain countries where physicians do not as yet recognize the value of the new diagnostic measures.

In our description of the various clinical forms of estivoautumnal fever, we shall first speak of the simple, regular, intermittent fevers; next, of the irregular and subcontinuous; and, finally, of the pernicious fevers.

The estivoautumnal fevers of the regular type are of two kinds, tertian and quotidian.

As the historical outline above given indicates, previous to our investigations the intermittent fever produced by the estivoautumnal parasite was held by those who did not think it absolutely irregular to be chiefly of the quotidian type. The tertian of which we speak at the present day was considered to be the result of two subintrant attacks of quotidian fever. Even Marchiafava and Celli, in their earliest work, the one in which they first described the estivoautumnal parasite, believed that it was connected with fevers of a quotidian type. When, however, we had investigated more thoroughly the course of the temperature and the conduct of the parasite, we recognized the tertian type; and at the present time, after still further researches, we are able to state that this is the predominating and most important type of the estivoautumnal infections.

Regular Intermittent Estivoautumnal Fevers.

Estivoautumnal Tertian Fever.

This variety is clearly distinguished from the ordinary tertian, not only by the nature of the parasite, but by the clinical course as well; that is to say, by the temperature curve, and by the tendency to aggravation and to the approximation and conjunction of the attacks, which often gives a continuous temperature curve.

In the simplest and most distinctly periodical cases the characters of the fever are as follows: It begins with an abrupt elevation, which usually reaches or goes above 40° C. (104° F.); this is followed by a period of oscillation in the thermic curve of perhaps more than one degree Centigrade. Of these oscillations, the most notable one is that preceding the crisis, in which the temperature is again raised usually to the highest point, after having been preceded by a marked lowering of temperature with temporary improvement of all the symptoms. In some cases the diminution of the fever which precedes the final elevation is so marked as to seem like the true crisis to one not familiar with this peculiarity of the estival tertian. There are five stages in the temperature curve of this form of tertian: (1) the febrile invasion; (2) the status febrilis, or fastigium, in which we sometimes note marked oscillations of the temperature; (3) a pseudocrisis; (4) the precritical elevation; and (5) the crisis.

The complete attack is apt to be of long duration; as a rule it lasts over twenty-four hours, and may go on to thirty-six or forty hours, so that the period of apyrexia between two attacks is only from eight to ten hours in length, and allows of no abatement in the headache, the prostration, and the gastric disturbances, the patients believing and asserting that the fever is continuous. This is the

course of the fever in typically regular cases, in which the regularity may be such that the curves of two successive attacks are identical as to the hour of invasion, the oscillations of the period of status, and the time of defervescence.

The differences in the temperature curves of this variety of tertian and the ordinary tertian are at once evident, and will be seen to be clearly demonstrated in the accompanying charts. They are so marked that, in view of the classical definition of the tertian, we cannot apply that name to the febrile curve described; in fact, on the day intermediate between the attacks, there is not that "perfecta integritas" which is often, although not always, found in the days of

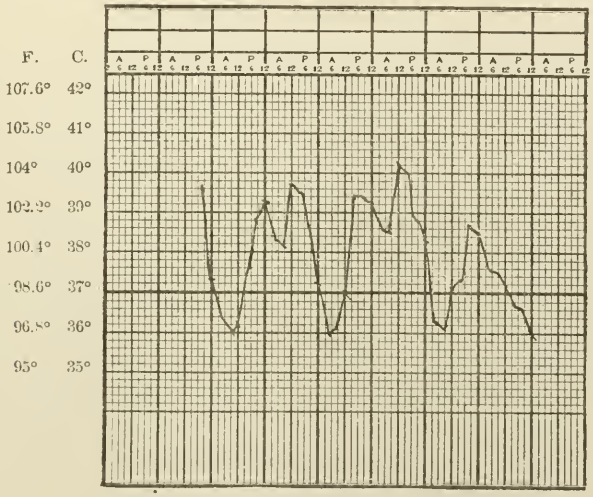


CHART No. 10.—Estivoautumnal Tertian.

apyrexia in the ordinary tertian; the attack is usually prolonged into a good part of the day interposed between the end of one attack and the onset of the next one, so that there is no day of complete apyrexia. But the single attacks begin every third day, which is the fundamental characteristic of the tertian type, and are prolonged into the next day. The estivoautumnal tertian, then, is a tertian with prolonged attacks (see Chart No. 10).

It is quite true that in the ordinary tertian we may have a similar curve, but this occurs only under special and altogether exceptional conditions, as when in a double tertian the two attacks approach so closely as to become united, giving a curve like that of a prolonged attack with two marked elevations, each of which, of course, represents a tertian attack. But in this case the form of the prolonged attacks results from the fusion of two attacks corresponding to the

maturing of two generations of parasites, which multiply at an interval of a few hours only; while the curve of the estival tertian represents only one attack with the peculiarities given above, and corresponding, as we shall see, to peculiarities in the development of the parasites. Thus the curve described is typical of the estivoautumnal tertian.

But both in the course of the single attacks of this tertian fever and in the complex course resulting from the rapid succession of the same, we may find certain variations consisting respectively in modifications in the curve of the attacks and modifications in the succession of the attacks.

The chief *modifications in the temperature curve* of the attack are: (*a*) the absence of a distinct initial elevation, causing it to lose its individuality and to become merged in the general temperature curve; (*b*) the absence of a distinct critical elevation, so that the curve of the attack by the disappearance of its various oscillations tends to be continuous, especially when the attack is relatively short; (*c*) an exaggeration of the pseudocrisis in such a way that the attack almost loses its individual characteristics, and there seem to be two quotidian attacks grouped in pairs. An examination of the blood, however, together with a study of the whole temperature curve, will show that every third day there is complete apyrexia, while on the intermediate day the temperature remits and for a little while only reaches 37° C. (98.6° F.); (*d*) the brief duration of the attack, which lasts only eight, ten, or twelve hours, giving us a curve similar to that of the simple tertian. Cases of distinct tertian fever with estivoautumnal parasites have been observed by Hewetson and Thayer, by Mannaberg, and once by us. In these cases there was one day of complete apyrexia; the attack began with a severe chill and ended with profuse sweating, and without an examination of the blood it might easily have been mistaken for the simple tertian due to the tertian parasite described by Golgi; (*e*) prolongation of the attacks, which usually is accompanied by an exaggeration of the oscillations of the period of status.

The modifications in the succession of the attacks are: (*a*) anticipation of the attacks, which may occur in cases tending to become pernicious in their nature, as well as in the relatively milder forms; (*b*) delay in the attacks, which can occur in the grave forms of the disease; (*c*) the presence of slight oscillations of temperature in the period intermediate between two attacks; (*d*) reduplication of the attacks (double estival tertian).

The above-mentioned modifications, especially if several of them occur in one case, cause curves so irregular that it is difficult and

sometimes impossible to understand them properly. An examination of the blood will often explain the irregularity. But even for other reasons, outside of those furnished by an examination of the blood, we are led to consider these irregular curves as belonging to the estival tertian type of fevers. In the first place, the complex curve with the attacks separated by incomplete apyrexia is often observed in primary affections,* while it often happens that the fever is regular and typical in relapses; in these, however, it may become complicated with one of the above-mentioned modified forms. In the second place, a febrile curve with prolonged attacks separated only by incomplete apyrexia may become regular after the dose of a certain amount of quinine, provided that this be not repeated. In the third place, the examination of many thermoscopic tracings shows the existence of all the transitional forms between a typical attack and the irregular varieties, so that we may suppose that the latter can return to the fundamental type of the estivoautumnal tertian.

Symptoms.—The febrile attack of the estivoautumnal tertian as well as that of the quotidian is accompanied by practically the same symptoms as those already described for the ordinary tertian and the quartan. We can distinguish three stages in the attacks of the estivoautumnal tertian (although not invariably, and not always as clearly as in the quartan and the ordinary tertian): (1) The stage of cold, (2) the stage of heat, and (3) the stage of defervescence and sweating. To the description of these stages, which we have already given in reference to the other forms of malaria, we shall add a few special characteristics of the fever under discussion. The development of this type of disease is frequently preceded by one, two, or even more days of discomfort, headache, a tendency to become easily tired, etc. The chills are sometimes absent, sometimes slight and of short duration, but they may be intense and of long duration in the regular intermittent forms. The accompanying symptoms are headache, which may be severe, pain in the limbs and body, especially in the lumbar region, prostration, epigastric pain, a coated tongue, intense thirst, nausea, vomiting, diarrhœa, and insomnia. The pulse is frequent and often dicrotic, and respiration is labored. The patient, whose face is red and whose eyes are injected and bril-

* This fact was well known to the older writers. Thus Sydenham wrote "Cum præmature, mense julio, intermittentes autumnales ingredientur atque increbrescunt, non statim genuinum typum inducunt quod intermittentibus vernis solemne est; sed continuas febres ita per omnia imitantur, ut nisi castigatissime utrasque examine trutinaveris, ab invecem discriminari non possint, et retuso paulis per constitutionis impetu, et frenata vi, jam in typum regularem migrant."

liant, is often extremely nervous and complains of great suffering, especially in the head. In the remissions of the fever all these symptoms decrease in violence; during the stage of apyrexia they may entirely disappear and be replaced by a feeling of well-being which is transitory and deceptive; sometimes they are merely attenuated, and the headache, although less severe, remains to cause the chief suffering of the patient. The splenic tumor is a constant symptom, and may be felt by palpation as early as during the second paroxysm. The sweats at the end of the attack behave much as do the chills; sometimes they are absent, sometimes slight, but they may also be copious and prolonged. The attacks in the tertian usually begin late in the afternoon, last all the following day, and end in the night or in the early morning hours of the third day.*

Examination of the Blood.—At the acme of the attack we find the small, non-pigmented amœbæ, which are discoid or annular in shape, motionless or else actively motile, the size of one-eighth or even one-fifth of a red corpuscle. The ones which are moving rapidly take on the most bizarre shapes; from the discoid or annular forms they may return to amœboid movements again. These non-pigmented bodies may be seen throughout the whole or nearly the whole of the febrile attack. With the advent of apyrexia the parasites begin to become pigmented by fine granules of pigment collected at the periphery, and to increase in size; so that we then find amœbæ as large as a quarter or a third of the red corpuscle, pigmented and of various shapes. The discoid bodies have dentate outlines, and the motile ones take on strange shapes with serrated contours, and are grayish in color. The red corpuscles containing the parasites are either normal or shrunken and wrinkled, and of a dark yellow color, being therefore

* Some sharp criticism has been aroused by our views in regard to this febrile type. It has been said that we have created new clinical types based only upon incomplete parasitic researches, and that we are in error in adopting the name of malignant tertian, because we force the meaning of the word tertian to describe a type quite different from that to which practice has consecrated the term. Now it is of great importance to observe that the clinical differences between the two forms of tertian were recognized by the older physicians. We have already quoted from Sydenham. We will also quote from Aulus Cornelius Celsus in regard to the two clinical types of the tertian. "Tertianarum vero duo genera sunt: alterum eodem modo, quo quartana et incipiens et desinens, illo tantum interposito descrimine, quod unum diem præstat integrum, tertio redit: alterum longe perniciosus, quod tertio quidem die revertitur, ex octo autem et quadraginta horis, fere sex et triginta per accessiones occupat, interdum etiam vel plus vel minus, neque ex toto in remissione desistit, sed tantum lævius est." From these words of Celsus, it is easy to see that he recognized another tertian besides the ordinary kind, with prolonged attacks of greater gravity, which evidently corresponded to our estivoautumnal tertian.

called "brassy bodies"; they always increase in number just before a new attack. We also find other endoglobular parasites as large as a third of the red corpuscle, round, non-motile, with a mass of pigment in the centre or at the periphery. Around them we sometimes find the residual hæmoglobin of the red corpuscle, the latter being decolored at its periphery. When we see these bodies in the blood, and some brassy bodies with parasites, we may predict the near approach of a new attack which will coincide with the maturing of a new generation of parasites. In fact, although, as we have already said, we rarely see fission forms in blood taken from the finger, when we find adult bodies containing granules of pigment and with pigment massed in the centre coincidently with the presence of parasite-containing brassy bodies, we may be very certain that fission is about to take place or has already occurred in the internal organs. This is demonstrated by the examination of blood taken during life from the spleen, and by the presence of the parasitic contents of the various organs in those lethal cases in which, while the peripheral blood contains pigmented or non-pigmented parasites, many of them within brassy bodies, the pulp of the spleen, the cerebral capillaries, and those of the intestinal mucosa, the abdominal fat, etc., contain many sporules. The fission forms are smaller than those of the ordinary tertian and the quartan. The number of spores varies from twelve to thirty, rarely exceeding or falling short of these limits, and is usually from twelve to twenty-four.

At the beginning of a fresh attack very few and sometimes no parasites are found in blood from the finger, while we may find many pigmented leucocytes. When the attack is in an advanced stage the young amœbæ return, increasing in number as the attack continues, and complete the cycle of their existence as already described. This life cycle, with sporulation frequently concealed if the examination be limited to blood taken from the finger—this cycle, which may be called "pyrogenic," because it is in relation with the various phases of the febrile paroxysms of the estivoautumnal tertian and with the succession of attacks, lasts about forty-eight hours.

In addition to the facts just related, others worthy of attention appear from an examination of the blood; there are cases in which, at the beginning of a new attack or even lasting well into it, we have the presence of adult forms within the brassy bodies, in which the pigment is distributed or gathered into a little mass; their number varies, but is most abundant in prolonged attacks. It sometimes happens that in an advanced stage of the attack we have a reappearance of adult forms in blood from the finger, from which they were absent at the beginning of the attack, and even, but rarely, forms in

which fission has begun and bodies which are the results of fission; these last, especially in grave cases and in prolonged attacks, may persist up to the precritical elevation of temperature.

The last-mentioned phenomena lead us to believe that the sporulation of the parasites takes up some time, and occurs successively and by groups; whence the prolonged attack in estival tertian and the variety of elevations observed in the curve, including the precritical, which would appear to correspond to the sporulation of the last group.

It has already been stated that the non-pigmented phase of the new generation lasts throughout or nearly throughout the attack; now there are cases in which the pigmentation may be delayed, and others in which it is precocious; that is to say, in these last we see the amœba develop and become pigmented even before the attack is ended, during the precritical elevation of temperature and the crisis. Precocious development is usually seen when the attacks tend to anticipate.

Finally, in the period preceding the new attack, in which the adult forms are found alone or in great number, we may also find young bodies which gradually decrease in number, while the pigmented bodies go on increasing.

In the mild forms of estivoautumnal tertian, the number of parasites is usually small, so that we do not succeed in observing the whole cycle of parasitic life. Because in these cases we usually obtain negative results at the onset and during the first hours of the attack, and the new generation appears only during an advanced stage, it follows that the relatively largest number of parasites is found during the apyrexia. In light forms of fever, which are often irregular from the presence of abortive or incomplete attacks, and which are very difficult to study, we may find no parasites at all in the blood for as long as twenty-four hours; but although the parasites are absent, we sometimes find a few pigmented leucocytes circulating in the blood, which in itself will suffice for the diagnosis of malaria.

If the infection have already lasted some time—usually in eight, nine, or ten days, rarely sooner or later—we find the crescent bodies appearing in the blood of the peripheral capillaries, and sometimes all the other phases of the endoglobular development of this form; and also those derived from it, such as the round ones with the pigment accumulated in the centre, and the flagellate bodies, which, as we have already said, are not formed in the circulating blood, but in the preparations, fifteen to twenty minutes after they have been made. We further note that three or four days before their appearance in the peripheral blood, the crescent bodies are found in the various

phases of their development in blood taken from the spleen. The crescent bodies increase with every febrile attack, and remain for a variable length of time after the fever has disappeared and the parasites of the pyrogenous cycle have gone. It goes without saying that the crescents do not appear in the blood if the febrile attacks are promptly prevented by sufficiently large doses of quinine; but if this remedy is given too late it would seem to facilitate the transition of the parasite through this stage, and the crescent bodies appear and persist, while the other forms of parasites disappear. This is also demonstrated by observations made upon bone marrow in some cases of pernicious fever which ended fatally after the disappearance of the parasites had been caused by the administration of quinine. We have already stated that the marrow of bones seems to be the chief seat of the formation of crescent parasites. Now in the cases referred to, while few or none of the fever-producing parasites were found in the viscera, many crescents in every phase of their endoglobular development were found in the marrow of the short and flat bones, and in that of the long ones which had become red. These crescent bodies are found in the red corpuscles which have lost their nuclei, but are never seen in the nucleated red cells, even when the medullary substance is rich in them. In lethal cases of pernicious fever, when the malarial infection is not of long standing, and in which therefore the hæmatoblastic transformation of the yellow marrow of the long bones is only beginning, we may find well-developed crescent bodies.

Estivoautumnal Quotidian Fever.

The estival quotidian, which is to be carefully distinguished from the quotidian of tertian and quartan origin, of which we have already spoken, may be regular as to the resemblance of the attacks to each other in regard to the hour of invasion, their duration, the elevation of the temperature, and the symptoms which accompany them. In a typical quotidian the attack is usually short, lasting six or eight, rarely twelve hours, and is composed of only one elevation of temperature, without any special oscillations. The elevation of the temperature is apt to be noticeable more for its rapidity and abruptness than for its duration. During defervescence the temperature, as we have before remarked, is notably lowered, usually to 35° C. (95° F.), and even lower (see Chart No. 11).

The quotidian soon loses the regularity of its course, because the attacks become prolonged, anticipate, or delay. The tendency to subcontinuity by prolongation, anticipation, and hence fusion of the attacks, is usually symptomatic of gravity of the disease. But there

are some cases in which, although the symptoms are not grave, yet the attacks are prolonged, and the periods of apyrexia are therefore brief and incomplete. On the other hand, in cases in which the attacks are quite distinct and regular, the disease may suddenly become aggravated. Delayed attacks occur in mild cases.

The *symptoms* which accompany quotidian fever are, as a rule, the same as those described for estivoautumnal tertian. The initial chill usually occurs, but it is less severe than that of the ordinary tertian and the quartan fevers. The headache, the pain in the limbs and in

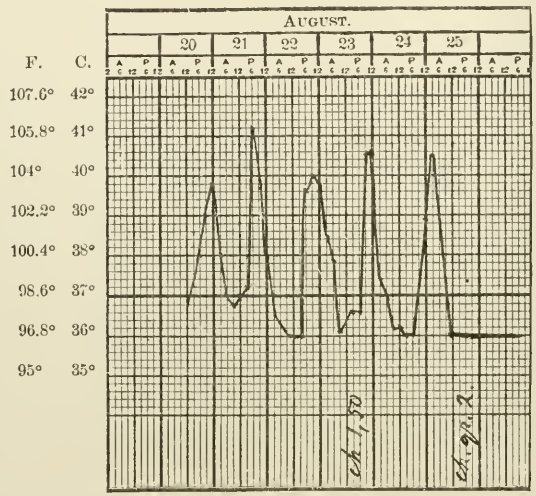


Chart No. 11.—Estival Quotidian Fever, showing the subnormal temperature in the period of apyrexia.

the lumbar region, and the gastrointestinal disturbances vary in severity according to the case, being generally less than in the estivo-autumnal tertian.

The quotidian is frequently a mild fever, and may be cured spontaneously; but it may be grave and even pernicious, although less frequently so than the tertian. A spontaneous cure usually occurs by attenuation of the attacks; this may take place gradually and with regularity. In some cases we see the apyrexia becoming more prolonged in its duration, the attacks shorter, the rise of temperature less marked, although the acme of the fever continues to come daily at or about the same hour. But the attacks may cease spontaneously even when, from the fact of their prolongation, it would seem that the disease was becoming aggravated. This resembles what is sometimes seen in the ordinary tertian, in which a spontaneous cure can occur even after a long and severe attack.

If the quotidian fever is not treated with quinine, it may last for several days in succession. If, as frequently happens in the quotidian, the attacks come in the evening and are prolonged into the night, the fever intermits in the morning, or only remits as it does in subcontinuous fevers, this being due to prolongation of the quotidian attacks. In this event, unless an examination of the blood is made, it is easy to make an error of diagnosis, especially in relation to typhoid fever, and more particularly when, in addition to an enlarged spleen, we have gastrointestinal disturbances. We recall several such cases, in which an examination of the blood gave a sudden revelation of the true nature of the disease.

The quotidian type is apt to be less distinct in primary affections than in relapses; indeed, the fever is not rarely subcontinuous in the former, and quotidian intermittent in the latter. This fact is well demonstrated by the autumnal and winter relapses of infections contracted respectively in the summer and the autumn, in which relapses the quotidian type is frequently and clearly manifested.

Examination of the Blood.—When this is made during a high elevation of temperature, we find a variable number of red corpuscles containing the small amœboid parasites which are more or less actively motile, or immotile in the discoid and annular forms. The same conditions obtain during the sweating period. During the stage of apyrexia the parasites become enlarged and pigmented, while their motility tends to diminish; they are thus changed into very small bodies, endowed with torpid movement, containing fine pigment granules at their periphery, or into immotile bodies containing very fine particles of hæmoglobin or fine pigment granules. Following these, we find parasites which are larger than the former, round and immotile, with small central or peripheral masses of pigment; and red corpuscles which contain parasites, are shrunken, wrinkled, and of a color like old brass—hence called "brassy bodies." The presence of these last-named bodies indicates the imminence of a fresh attack. In the quotidian, multiplying forms of the parasites are rarely or never seen in peripheral blood, their multiplication occurring in the blood of the internal organs (spleen, bone marrow, cerebral capillaries, etc.). When the new attack has fairly begun, the young non-pigmented parasites are again seen in blood taken from the finger, for they represent the new generation which is commencing the cycle of existence already described. This cycle is completed in about twenty-four hours, as may be ascertained by comparing the parasitic data with the course of the fever.

When the fever departs from a typical course, it becomes much more difficult to follow the development of the parasites in their

various stages. Thus in the quotidian which becomes irregular from subintrance of the attacks, the contents of the blood become more complex, as at every period of the fever we find parasites in their various stages of development, with a predominance, however, of the form corresponding to each particular phase of the fever. If the attacks become irregular by reason of their prolongation, even to the point of a complete omission of the period of apyrexia, the contents of the blood are similar to what we find in a typical quotidian. In the lightest forms of quotidian, the parasitic contents of the blood may be very scanty in amount, and in some cases for even a considerable length of time may be altogether absent. But even in these non-parasitic periods we are apt to find a few pigmented leucocytes. When a spontaneous cure occurs, the parasites become progressively rarer towards the later attacks, and may altogether disappear in the last abortive ones. In other cases crescent bodies take the place of the amœboid bodies. This is also the case in the spontaneous cures occurring in estivoautumnal tertian fevers.

Irregular Intermittent Fevers.

We have seen that in the quartan and ordinary tertian infections there may be irregular intermittent fevers, which, by the presence in

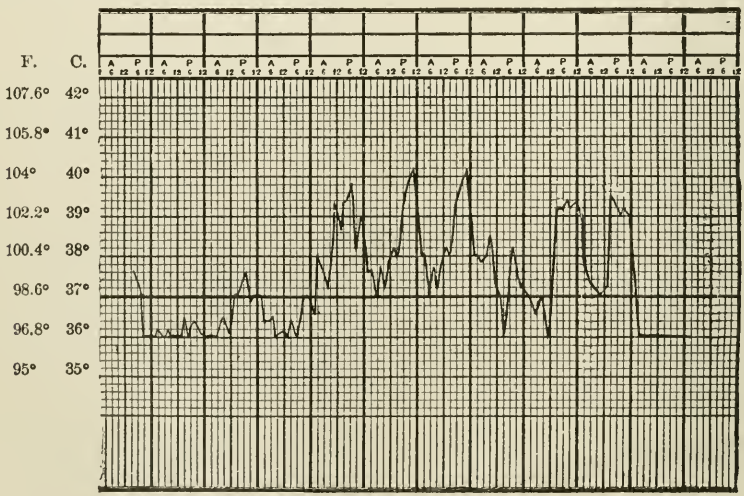


Chart No. 12.—Irregular Estivoautumnal Intermittent.

the blood of the quartan or tertian parasites, and by the absence of complications, are shown to be the result only of these infections.

In estivoautumnal infection the irregular intermittent fevers

(charts Nos. 12 and 13) are of much more frequent occurrence; the manner in which the irregularity occurs, whether by a modification

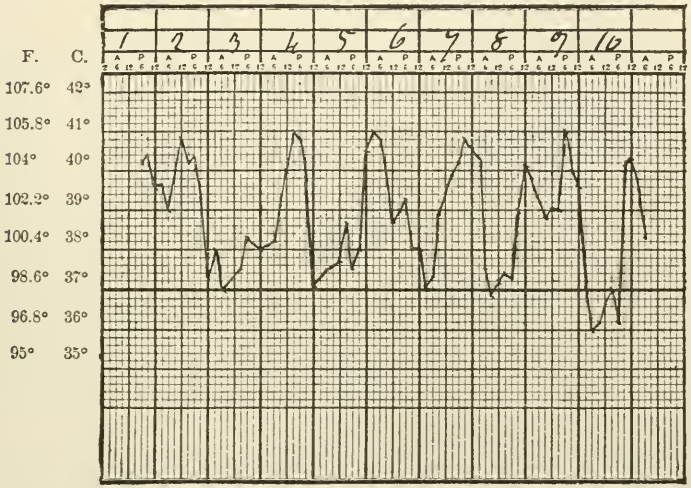


CHART No. 13.—Irregular Estivoautumnal Intermittent of Tertian Origin, with Anticipating Paroxysms.

in the curve of the attack or a modification in the succession of these attacks (in both quotidian and tertian), has already been described. We would further state that there are some irregular intermittent curves whose origin from a regular type it is not easy to recognize. We see a series of attacks which begin at different hours, have a variable duration, and variable modifications of the curve. As to the duration, the difference between one attack and another may be of several hours; for instance, an attack lasting forty-two hours may be followed by one of nineteen hours, the latter by an abortive attack, and this by one lasting as long as did the first (see chart No. 14).

Among these irregular forms are some which are such only as regards known types. Thus we have several times observed a febrile type in which the attacks were repeated at intervals of thirty-six hours; for instance, while the first attack began at 6 A.M. the second began at 6 P.M. of the following day, the third at 6 A.M. of the fourth day, and so on. This type of fever, which is neither quotidian nor tertian and might almost be called *subtertian*, may go on in the same way through several attacks (see chart No. 15).

Without dwelling longer upon the irregular intermittent course of these fevers, of which many and varied examples are met with in practice, we are inclined to ask ourselves if there is, corresponding to these irregular fevers, a species or a variety of parasite whose life cycle has the characteristic of irregularity, whence the irregularity of

the febrile type. In these fevers, however, we find the same parasitic forms as those described in the quotidian and tertian infections, viz.,

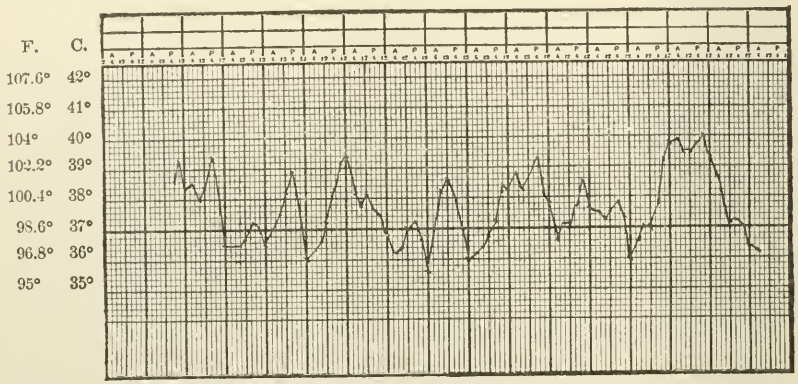


CHART No. 14.—Irregular Fever of Estival Tertian Origin. On examination of the blood the estivo-autumnal parasites only were found.

those of the pyrogenous cycle and the crescent bodies destined to undergo their further development outside of the organism. At the acme of the fever the non-pigmented motile or annular bodies predominate; towards the end of the attack the bodies containing particles of pigment; near the time of the new attack the parasite-contain-

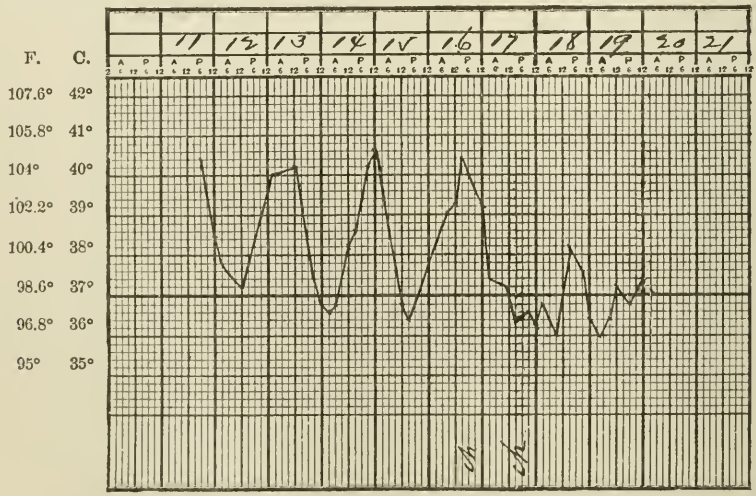


CHART No. 15.—Irregular Estivoautumnal Fever. The maxima of the individual paroxysms are separated by intervals of about thirty-six hours.

ing brassy bodies. The crescent bodies, as regards the time of their appearance, their aspect, etc., behave in the same way as described when speaking of the tertian and quotidian fevers.

Now if the parasites are the same as those found in the typically regular fevers, what is the cause of the irregularity? It seems to us that this cause can be found only in the variability in duration of these same parasites, and this follows logically from what we know about the various phases of their development in relation to the febrile manifestations. That the duration of life of the malarial parasite may vary is also a known fact, as we have seen in the case of quartan and ordinary tertian fevers. There we observed precocious or retarded sporulation, causing (if the occurrence be not an isolated one) the anticipated or postponed attacks, and also the irregular ones. This variability is without comparison greater in the parasites of the estivoautumnal fevers, not only in different patients, but also in the same patient, for which reason we may see an alternation of quotidian or tertian and irregular attacks.

Irregular fevers appear to vary in frequency according to the climate, and in the same climate according to the season. Perhaps this is the chief reason why several writers have given the denomination of *irregular fevers* to those of the estivoautumnal species. We have stated elsewhere why such a nomenclature was impossible of acceptance.

Subcontinuous (Remittent) Estivoautumnal Fevers.

We have seen how the quartan and simple tertian fevers become subcontinuous by modifications occurring in single as well as in a succession of attacks. Even more easily and frequently does this happen in the case of the intermittent estivoautumnal fevers.

We prefer the name *subcontinuous* to that of *continued* or *remittent* malarial fever, and in this Baccelli agrees with us, the term subcontinuous being used conventionally by many physicians to express the fact of the continuity which proceeds from a fever essentially intermittent.

We rarely have occasion to observe the course of the temperature in these fevers—the prolonged curve of a genuine malarial fever in which the single attacks are not separated by complete apyrexia—because as the disease is frequently grave in its nature, and as we possess the specific remedy for it, the prompt administration of the latter usually cuts the fever short. Nevertheless we can form an idea of the manner in which malarial fevers become continuous by a study of the course of fevers of the less severe type, in which we note the tendency of the attacks to prolong themselves or to anticipate.

Subcontinuity can originate from the estivoautumnal fevers in the following ways: (1) *By prolongation of the attacks.* If the prolonga-

tion be such that the attacks run into each other, a continuous fever is the result, the original type of which (especially in the case of the tertian) it will be difficult to recognize from a mere examination of the temperature curve, and it will also be difficult to distinguish the separate attacks from each other and the elevation of temperature of the onset from that of the precritical stage. (2) *By anticipation and subintrance of the attacks.* The onset of one attack occurs before the preceding one is completed, and, in the tertian, we see a complex curve with a series of closely approximated elevations during the twenty-four hours, corresponding to the thermal oscillations proper to the febrile attacks. (3) *By duplication of the attacks.* We can easily understand how in the tertian, the attacks of which are of such long duration, duplication will cause continuity almost by superposition of the attacks, giving a complex curve in which it is almost impossible to recognize the original type. In the rare cases of simple tertian caused by the estivoautumnal parasite, it will be easy to recognize duplication of the attacks. Subcontinuity by prolongation of the attacks is frequent in quotidian fevers.

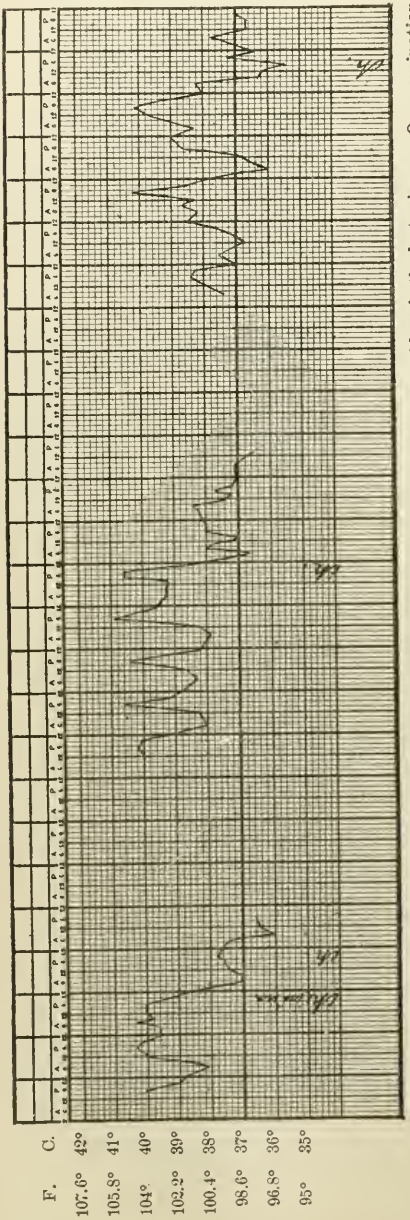


CHART No. 16.—Subcontinuous Relapsing Malarial Fever of Estival Tertian Origin. This origin is made evident in the last relapse. On examination of the blood the estivoautumnal parasite alone was found.

While the transition from an intermittent to a subcontinuous fever with the gradual obliteration of the periods of apyrexia is frequently observed by the physician, much more

often will it be given him to see a fever subcontinuous from the beginning, and to recognize its origin only when, spontaneously or under the influence of the specific remedy, it becomes intermittent.

A recognition of the genesis of subcontinuity is easy when it occurs in the quartan and tertian fevers, because it is led up to by symptoms characteristic of the onset and the termination of the attacks, because of the greater simplicity of the temperature curve, and because of the fact that the parasites complete their whole life cycle in the circulating blood, and hence can be readily observed. But as in the estivo-autumnal fevers these symptoms are often wanting, and the curve of the tertian is already complex in itself, and the parasites complete only a few phases of their life cycle in the circulating blood, it is not usually possible to recognize the original type from which the subcontinuous fevers originate, especially if they are subcontinuous from the onset. These subcontinuous fevers may be relatively mild, or they may be grave and even pernicious in their nature.

Their duration depends nearly always upon the treatment; if the specific remedy be promptly administered, the fever usually remits and ceases with promptitude, or it may intermit, to resume again for a short time, and then to terminate definitely. Thus, their duration is usually four, five, or six days, or a week at the most; but if they are not treated at all or only unskilfully, they may be prolonged for one or several weeks. In the latter event they may remain relatively mild, producing a progressive anæmia of variable severity; or they may gradually become aggravated and suddenly take on the aspect of a pernicious infection, as will be seen in one of the case histories given later in this section.

Previous to the discovery of the malarial parasite, and even afterwards before it was the custom to examine the blood, many forms of fever were included among the subcontinuous malarial which in reality had no connection with the infection, as is easily proved by their symptoms, their long duration in spite of the administration of quinine, and the data given by pathological anatomy. It is a well-known fact that physicians practising in malarial countries have a tendency to mistake for malaria infective diseases of quite a different nature (irregular typhoid, febrile icterus, cryptogenic septicæmia) or to regard it as a complication in other diseases (pneumonia, typhoid fever, etc.).

Symptoms.—The clinical course of the subcontinuous fevers, as we have already said, has not been studied in detail because the prompt cure effected removes the opportunity of so doing. Thus we are no longer permitted to observe those transitions from the intermittent to the subcontinuous fevers so classically described by Torti and other

physicians following in his footsteps, except in those few exceptional cases in which the fever continues in spite of the treatment, although we may perhaps suspect it from the history of the patients. In patients suffering from subcontinuous fever, we find the same symptoms as in the intermittent fevers, but of greater gravity. In the subcontinuous fevers originating as such, the patients complain of prodromes lasting one or two days, such as a sense of discomfort, pain in the whole body, weakness, and loss of appetite, after which comes the fever unaccompanied by chill, or with merely a slight sensation of chilliness. When the fever has lasted two or three days, the skin is dry and sometimes slightly subicteric in hue, the eyes are reddened and shining, the patients are greatly prostrated and restless, the headache is severe and often so intense as to force groans from the patient, and there are also pains in the lumbar region and in the limbs, which are increased by pressure. The tongue is coated, and thirst is intense; vomiting is frequent, often caused by the great amount of water taken, and is accompanied by epigastric distress and perhaps diarrhœa occasionally associated with tenesmus. At night the patients are restless and anxious, their sleep being interrupted by a wakefulness characterized by agitation and even slight delirium. The pulse is rapid, compressible, frequently dicrotic. The urine is highly colored and contains much sediment. A physical examination shows that the spleen is enlarged in size, the degree of the enlargement depending upon whether the infection is primary or whether the patient has previously suffered from malarial diseases. In the first instance as early as the third day the spleen is found to be enlarged, and even prominent. Careful examination will also show slight enlargement of the liver. At a more advanced stage, we often find an increase in the area of cardiac dulness, especially on the right side.

The course of the *temperature* can be partly inferred from what has already been stated. We have described the modifications of the intermittent fevers which lead to subcontinuity; from these modifications it is not difficult to reconstruct the resulting curves, the most simple of which are those due to prolongation, the most complex those due to overlapping or to duplication of the attacks. As a rule we have during the course of a subcontinuous fever marked remissions with alleviation of the symptoms, occurring after the temperature has been elevated for a variable time, even one or two days with slight oscillations; or these remissions may be more frequent and more irregular, followed by a rise as high as 41° C. (105.8° F.) with an exacerbation of all the symptoms. The fever may, however, have a regular course, as in the subcontinuous of quotidian origin by prolongation of the attacks, which, as we have already remarked, may go on

with morning remissions and evening exacerbations quite like a continued fever.

Examination of the Blood.—This demonstrates a complexity in the parasitic contents of the blood as complex as that of the temperature curve. The parasitic contents may be divided into three classes. There may be one generation only of the parasites in which the multiplication of the bodies goes on for several hours longer than is usual in the case of intermittent estivoautumnal infection; or there may be two generations of parasites; or at the several periods of the fever we find amœbæ in various stages of development so that it is quite impossible to distinguish and follow the evolution of the different generations.

Whether in this complex fever there are two generations of parasites, or whether there is only one of which the adult forms continue to multiply, periods in which the result of an examination of the blood is negative are not apt to occur as they do in the typical tertian. The presence of the parasites is constant, although their numbers may vary; whence the ease of diagnosing malaria even in cases in which the clinical assistance given by the occurrence of intermissions is absent. It is unnecessary to add that in the subcontinuous fevers the parasites are the same as in the intermittent estivoautumnal fevers, including the crescent phases.

The subcontinuous fevers have been subdivided, even by recent writers, in various ways according to the predominance of certain symptoms; thus, gastric subcontinuous fever, rheumatic subcontinuous, bilious subcontinuous, typhoid subcontinuous, pneumonic subcontinuous, hæmoglobinuric subcontinuous fever, etc.

Typhoid subcontinuous, or *remittent, fever* means a subcontinuous fever in which the totality of the symptoms is such that unless a proper valuation be made of the previous symptoms and history of the patient, the physician may be easily led to a diagnosis of typhoid infection. Every sign of the latter may be present; headache, epistaxis, the abdominal symptoms, the splenic tumor, prostration, cerebral phenomena, roseolar eruption, etc.; in a word, a complete clinical picture of typhoid fever. A knowledge of these clinical types is of the greatest importance, because without an examination of the blood, physicians even at the present time may be led to an erroneous conclusion; in other words, to make a diagnosis of typhoid infection. The opposite mistake is sometimes made in malarial countries, namely, that of regarding typhoid fever with a somewhat abnormal course to be a typhoidal remittent fever, and this even in spite of the fact that quinine given in such large or long-continued doses as to cause symptoms of poisoning as severe as amaurosis, is absolutely in-

efficacious. As early as 1866 the attention of Baccelli was called to the typhoid subcontinuous fever, and he made known the clinical data by which the true malarial nature of the disease might be recognized through the mist of deceptive symptoms, and be differentiated from real typhoid fever. These symptoms are the frequently intermittent onset, high temperature from the first day of the disease, the initial subicteric tint, the rapidity of occurrence of the cerebral phenomena, etc. At the present day, in addition to careful observation of the symptoms, a microscopical examination of the blood on the first day of the disease will enable us to establish the diagnosis.

In bilious or icteric subcontinuous fever we have more or less marked jaundice accompanied by enlargement of the liver, bilious vomiting, and stools. This form may be confounded with febrile icterus, or "Weil's disease," especially when it is preceded by severe pain in the limbs which is increased on pressure.

We have already spoken of the *duration* of this fever, stating that when it is properly treated it does not last more than five or six days. But sometimes cases occur in which, in spite of the administration of quinine and the decrease or disappearance of the parasites, the fever continues for two, three, and even four days longer with a progressively milder course.

We have also said that the subcontinuous fevers may be of either a mild or a grave type. The grave nature of the disease may date from the beginning, or it may be established later and go on increasing to a fatal issue, unless overcome by the specific remedy. Thus in the typhoid subcontinuous, which goes on augmenting in severity, the tongue becomes dry, the pulse rapid, the senses dulled, agitation and delirium are followed by coma, and collapse precedes death. In the icteric subcontinuous fevers hemorrhages take place into the skin and the mucous membranes, and cerebral phenomena occur as in pernicious jaundice. In one case of icteric subcontinuous fever, in which the disease had lasted for four days and the coma two, there were found at the autopsy in addition to melanæmia and melanoses, and many parasites in the blood and the internal organs, diffuse necroses of the liver, and fatty degeneration of the kidneys, the heart, and other organs.

According to Torti, these grave subcontinuous fevers are called "solitary" pernicious fevers, that is to say, fevers which without perceptible intermissions become progressively aggravated and present a complex aggregation of symptoms, among which one or more of those characterizing the group of comitatæ may become more or less marked, without, however, predominating to such an extent as to form alone the clinical characteristic picture of the case.

Pernicious Fevers.

When, during the course of an intermittent or of a subcontinuous estivoautumnal fever a symptom of gravest import arises, the "*ferale aliquod et peculiare symptoma, morbo ipso, quam comitatur deterius*" of Torti, which dominates over all the other symptoms, and from which comes the danger to life, the infection is called complicated pernicious fever (febris comitata).

Pernicious infections in temperate climates like that of Rome occur in the summer and in the autumn, even late in the autumn if the cold weather be delayed. In the winter they sometimes occur as relapses, but altogether exceptionally as primary attacks, for pernicious forms are rare in cold weather. In the Roman hospitals, we rarely see a case of pernicious fever in the winter, whence the necessity for medical students of frequenting the hospitals at other seasons of the year if they wish to be well informed in regard to the grave forms of malaria.

The pure form of pernicious fever, that is to say, the kind in which malaria alone is the cause of the pernicious character of the disease, is usually preceded by other attacks which have either been untreated or mistakenly treated, and which have been of more than ordinary severity, as evidenced by the headache, vomiting, icterus, distress, prostration, etc.—symptoms which, in a more subdued form, persist during the stage of apyrexia. The cases in which the pernicious attack is said to be developed *d'emblée*, that is to say, as such without preceding attacks, as we shall see later, are due to complications, especially sunstroke. We have never seen a true pernicious fever in the first attack, but it should be added that we have seen some preceded by only two attacks or even one, and that pernicious attacks are more frequent in recent infections, as may be shown in lethal cases by the small amount of melanosis, and by the slight enlargement of the liver.

Complicated pernicious fevers may be classified according to the febrile type, or according to the paramount symptom. Following the first plan, we have tertian, quotidian, or subcontinuous pernicious fevers. Sometimes, although exceptionally, the fever may be lacking, the pernicious symptom being the only indication of the disease. These apyretic cases are called concealed or larval pernicious fevers.

The duration of the pernicious attack may be brief or protracted; when the former, it is about the length of the typical quotidian or tertian attack; when the latter, it may last two, three, or even more

days with a continuous fever, whose curve shows more or less marked remissions.

In intermittent pernicious fever there is only one attack if the case have been promptly and efficiently treated; otherwise there is a second, and very exceptionally a third attack; in the last case, unless fortunately prompt treatment be efficacious, the issue is always a fatal one. The symptom which gives the stamp of perniciousness to the case may appear either at the onset or during the course of an attack, as, for instance, at the time of the precritical elevation of temperature. After the crisis, or during the remission, this symptom may disappear, or more frequently is much attenuated, but it is ready to return with even increased severity in the next attack.

The appearance of the pernicious symptoms occurs either after premonitory symptoms, whose import does not escape the practised eye, or treacherously and with brutal abruptness. The latter is especially the case in old people and in persons suffering from diseases of the circulation; the coma may come all at once, without the slightest warning, and be followed a few hours later by death. We shall see presently how an examination of the blood, by its revelation of a large number of parasites, may enable us to foresee and frequently to avert the danger.

Pernicious infections were known to the earlier physicians, who have left us clear and detailed clinical descriptions of them, such as are of use to us at the present day. Especially valuable are those of Torti, in which this justly celebrated physician shows himself to be not only a wise and profound observer, but also a writer of precision and elegance.

The complicated pernicious fevers (comitatæ) take their name from the predominating symptom; this gives us varieties called comatose, delirious, tetanic, hemiplegic, choleraic, algid, dysenteric, hemorrhagic, etc. Some authors have attempted to classify them in groups. Thus Torti, starting from his conception of the nature of the fevers, divided them into two groups, the colliquative (choleraic, hemorrhagic, cardiac, diaphoretic), and the coagulative (syncopal, algid, lethargic). Some of the recent writers classify them according to the system or the organ from which the pernicious symptom develops. In describing the pernicious fevers, we shall dwell particularly upon those which occur the most frequently, first of all upon those whose symptoms emanate from the nervous system.

Comatose (*Lethargic*, *Apoplectic*) *Pernicious Fever.*—In this fever, which is the most frequent of all, the culminating symptom is the profound coma into which the patient falls rapidly or after prodromal symptoms, such as grave prostration, impaired memory, somno-

lence, severe headache, disturbed vision, stupor, or delirium. When in coma, the patients lie in the supine position and appear to be in a profound slumber. The pupils are contracted, occasionally unequal; the conjunctivæ are sometimes of a subicteric tint; respiration may be slow and quiet, or noisy, irregular, and interrupted by pauses, or it may be frequent and superficial. The pulse is usually slow, hard, and full at the beginning, but later, in cases that tend to a fatal issue, soft, rapid, and thready. Percussion shows dilatation of the right heart. Reaction to every form of stimulation is absent, the patients remaining irresponsive to injections of quinine and to all other excitants. The deep reflexes are sometimes present and sometimes abolished. Spasms of certain groups of muscles have been observed, but in other cases there has been complete paralysis of the muscles, or complete hemiplegia. Punctiform hemorrhages are frequently found on the face or on the conjunctivæ, and the ophthalmoscope often reveals them upon the retina as well. The coma varies in duration according to the case; sometimes it lasts a few hours, sometimes one, two, three, and even four days. It either resolves or ends in death. In the latter event, the respiration becomes periodically intermittent, or else short, superficial, and irregular, the pulse is thready, the color of the face is ashen, tracheal râles appear, a clammy sweat comes out over the whole body, which gradually becomes colder, and death occurs usually after a protracted struggle. In the case of resolution, the coma gives way to stupor and then to sleep; the patients begin to move about, open their eyes, and gradually regain consciousness. When they first begin to speak, there is frequently bradylalia, a certain scanning of their words, an insufficient motion of the lips and tongue.

Coma is sometimes followed by a delirium which may be quiet, or else be accompanied by so much agitation that the patients endeavor to get away, and have to be watched with the greatest care. The delirium may be of short duration, or it may last for days, ending in recovery or in fatal collapse. We recall a case in which coma was followed by a delirium which became progressively more and more severe, was accompanied by great agitation and frequent cries, lasted three days, and ended in death. But the coma may disappear with the crisis of the febrile attack, to return again with the next attack. In the interval between the two attacks, the patients are usually apathetic, stupefied, speak with difficulty and incoherently, and usually complain of severe headache. The coma of the second attack lasts longer and is less apt to resolve.

As to the course of the fever, this, as we have already said, may be intermittent or subcontinuous. In lethal cases the fever ceases a

few hours before death; or persists continuously, with slight oscillations, for three or four days; or ceases, only to return in very high degree a few hours before death, which then occurs during hyperpyrexia (41° C. = 105.8° F.—and even higher).

Comatose pernicious fever may be fatal in the first attack, after two or three febrile movements have occurred and after a coma which has lasted ten, twelve, or fourteen hours. Death may also occur after quinine has been freely administered of during the attack. But the most surprising fact of all is that death sometimes occurs during the second attack, when treatment was begun in the first; and also after two or three days of coma, even when the efficacy of the remedy is shown by the gradual diminution in the number of the parasites up to their total disappearance. In these cases we frequently find punctiform hemorrhages in the cerebrospinal substance. These might be called *comatose-apoplectic pernicious fevers.*

Delirious Pernicious Fever.—We have already mentioned the fact that in the comatose form delirium may precede and follow the coma; but in some cases the delirium is the predominant symptom, and assumes an acute form, which, after a variable interval, is followed by prostration and also stupor and coma. The delirium is sometimes preceded by a period of gay excitation, followed by hallucinations and furious excitement, in which the patient shrieks, tries to get out of bed, and even out of the hospital. In favorable cases, the delirium is followed by sweating and a deep sleep, from which the patient awakes to a condition of well-being; but death may occur during the attack, often preceded by coma of variable length.

Tetanic Pernicious Fever.—The patients are prostrated or delirious; they have trismus, contractions of the limbs, with or without opisthotonos; the abdomen is flattened; the eyes deviate. The muscular contractions vary in their behavior during the attack; they may become lessened without entirely disappearing; there may be exacerbations during which the rigidity of the trunk is increased and the pelvis is lifted up, and there may even be erection of the penis. The tetanic attack may slowly resolve, or it may end in death with a very high temperature.

Eclamptic Pernicious Fever.—This occurs chiefly in children. During the attack the patients are prostrated, and have attacks of general clonicotonic convulsions of variable duration, followed by an increase in the stupor or the coma.

To these pernicious fevers with their symptoms of cerebral irritation may be superadded others in which the patient has a truly *meningitic* aspect. This form also occurs more frequently in children and in young people. Without an examination of the blood, unless

we are already familiar with the history of the case, we may easily be led into an erroneous diagnosis. The patients have vomiting, a slow pulse, headache, rigidity of the back of the neck with pain on pressure; and then deep sleep, convulsions, vesical paralysis, marked superficial and deep hyperæsthesia; and finally the pulse becomes rapid and arrhythmic, and coma supervenes. This meningeal type develops rapidly in its various phases, and recalls the form of cerebrospinal meningitis with a hyperacute course.

But we also find forms of pernicious fever in which focal symptoms exist alone or in combination with the symptoms of diffuse cerebral affections just described. This gives us other varieties of pernicious fevers, as the *hemiplegic*, in which in addition to sopor we have hemiplegia, often in conjunction with hemianæsthesia; the *aphasic*, in which motor aphasia is observed alone, or in conjunction with hemiplegia or right brachial monoplegia; the *amaurotic*, in which the attack, among other grave symptoms (delirium, profound sopor, etc.), is accompanied by total blindness, which, as a rule, disappears with the disappearance of the fever but may persist for a variable time after the attack, and in some rare cases becomes permanent.

In the malarial infections we may, moreover, have a complexity of nervous symptoms, among which predominate those of cerebral or cerebrospinal disease, such as bulbar paralysis, acute ataxia, electric chorea, etc.; these infections are sometimes of long duration, and because of their gravity we include them among the pernicious fevers.

The form with *bulbar symptoms* is not infrequently met with in our hospitals, and has recently been several times described (Marchiafava, Bastianelli and Bignami, Orlandi, and others). When a physician unexpectedly encounters this disease, he is easily inclined at the first glance to think that the case is one of a patient with bulbar paralysis who has become infected with malaria, but this suspicion disappears after a careful examination and after seeing the gradual resolution of the symptoms. The chief symptoms are difficulty in articulation which may even reach anarthria, a weak and nasal voice, inferior facial paralysis often of one side only, a half-open mouth from which drools the saliva, a pendent lower lip, a dry and only slightly movable tongue, difficult or abolished deglutition. If the attack tends to a fatal issue, we have the added symptoms of sopor, a thready, intermittent pulse, labored and stertorous breathing, and clammy sweat. When, however, the result is favorable, the patient recovers from the more severe symptom as soon as the fever falls; the bulbar symptoms usually persist for some days, although in milder form, and then gradually disappear, the dysphagia going

first, then the dysarthria and nasal voice, and the paresis of the lower part of the face. Two or three weeks may elapse before resolution is complete. If the malarial infection have not been properly treated, we shall have an exacerbation or even a return of the bulbar symptoms in the relapses.

With these symptoms there are sometimes associated disturbances of equilibrium which recall the staggering gait of cerebellar disease. In a case of relapse, Bastianelli and Bignami noted an unsteadiness of gait as in drunkenness, diminution of strength on the left side, right facial paralysis, deviation of the tongue to the left, difficulty in speaking, nasal voice, grave prostration, and apathy. When the fever fell and tonic treatment was begun, these symptoms all disappeared within a few days.

Ataxic Pernicious Fever.—A grave form of malarial infection accompanied by nervous symptoms, among which those of acute ataxia predominate, has been described. To this class belong the cases of Angelini and Torti, who interpreted the group of symptoms as those belonging to multiple sclerosis. The valuable work of these writers has not been thoroughly understood by those who have deduced from it the argument that malaria is one of the causes of multiple sclerosis. A nervous, transitory, relapsing syndrome similar to that of multiple sclerosis should not be taken for a genuine form of this disease.

One of the cases of Torti and Angelini was that of a young man who had been infected by malaria three months previously. In a relapse occurring October 16th he had vomiting and vertigo. On November 8th the same symptoms reappeared. On the 9th he found articulation difficult, speech being slow and scanning; there was great weakness of the lower limbs, a vacillating gait with a tendency to fall forwards, exaggeration of the tendon reflexes, ataxia of the upper limbs, volitional tremor, slow pupillary reflexes, and slight nystagmus, but normal sensibility. On the following day the symptoms were all more marked; the vomiting was incoercible and rendered rectal alimentation necessary; there were extreme weakness, apathy, a feeble voice, complete muscular relaxation, increased dysarthria, vertigo, no matter what position was taken by the patient, and progressive anæmia—all of which gave the disease an appearance of the greatest gravity. Although the temperature was normal for several days, an examination of the blood was made, and estivo-autumnal parasites were found. The stimulant and specific treatment which was then immediately resorted to and continued for several days following, brought about an improvement in the condition which, on the 15th, was very marked in spite of the persistence in the blood of amœboid and crescent parasites. An ophthalmoscopic examination showed retinal hemorrhages. On the 18th there were only crescent forms, but these in increased number. The im-

provement was rapid, but the nervous symptoms disappeared slowly. On December 13th the patient left the hospital in good condition, with slight dysarthria. December 31st he returned to the hospital with fever, anæmia, and a syndrome similar to the one just described. In the blood there were estivoautumnal amœbæ and crescent bodies. When the fever dropped, the hypodermic injections of quinine (50 cgm. a day—gr. viii.) were continued, and there were diminished nervous symptoms and apyrexia until January 12th, when there was a light attack with a temperature of 37.8° C. (100° F.). On January 15th, 16th, 17th, and 18th there were quotidian febrile attacks, with high temperature, parasites in the blood, and exacerbation of the nervous symptoms. After eight days of apyrexia, there was a return of three quotidian attacks, in spite of the continued use of small doses of quinine. When the dose was increased the fever disappeared entirely, and the patient under good nutritional conditions had no return of the nervous phenomena.

Another case similar to the preceding, with a febrile malarial infection was observed by the same writers.

A youth of 22 years, anæmic, had suffered since August from fever with a few relapses. In the latter days of October he had slight vertigo, weakness, and an impediment in walking. On November 13th he was admitted to the hospital because of increased vertigo and headache. His speech was slow and scanning, he had nystagmus, a tremulous tongue, volitional tremor, and inability to walk without support, and was seriously anæmic; the temperature was normal. As all these symptoms continued and the patient's condition kept getting worse, the blood was examined, and the presence of estivoautumnal parasites was discovered. Quinine and arsenic brought about a speedy cure of the infection together with a disappearance of the nervous symptoms.

The study of these cases teaches us that active malaria—that is to say, malaria with parasites in the blood—is capable of giving a complex of nervous symptoms of great severity without fever, as was shown in the second case, or with but slight fever, as was shown in the first case in which an elevation of temperature occurred but once. These are examples of true *concealed or larval pernicious fever.*

A case of grave malarial infection during which the symptoms of *electric chorea* were manifested, was observed by Bastianelli and Bignami. The patient was a boy of 19 years, who came to the hospital to be treated for a grave fever, the nature of which was unrecognized during the first week. For several days the fever continued to be irregularly intermittent or remittent, and was prolonged for about twenty days with brief interruptions, accompanied by vomiting, diarrhœa, splenic tumor, great prostration, and dulness of the sensorium. During the fever the anæmia became very grave, and special nervous symptoms arose, consisting in clonic movements and abrupt and rapid rhythmical jerkings of the muscles of the shoulder, neck,

face, and eyes. Upon a day in which the patient's condition was most serious, an examination of the blood showed the presence of estivoautumnal parasites, and revealed the real nature of the disease. Prompt specific treatment caused cessation of the fever, but not of the symptoms of motor irritation, to which was superadded a muscular weakness so great that the patient could not lift his head from the pillow nor move his body. The muscles became rapidly atrophied without showing any qualitative alteration in their reaction to electricity, but merely a diminution in the galvanic and faradic excitability. At the same time the patient was in a state of mental confusion, with agitation and hallucinations, especially at night; following this, he had apoplectiform and epileptiform attacks which resolved without leaving a trace. After his illness had lasted about a month, the general condition began to be bettered, as was also sanguinifaction, and improvement in the nervous symptoms followed. A few relapses of the malarial fever, which were of no great severity, interrupted the course of recovery for about a month and a half. But these were overcome with the salts of quinine, and the patient advanced rapidly to convalescence; at the beginning of January, after about three months of sickness, he was completely restored to health. The parasites found in the blood both in the original infection and in the relapses were the estivoautumnal; the blood, moreover, showed the alterations of ordinary post-malarial anæmia.

Da Costa (quoted by Thayer) observed a case of paraplegia with volitional tremor, severe headache, bitemporal hemianopsia, and psychical symptoms with estivoautumnal infection. Specific treatment with quinine brought about a complete recovery.

Finally, in these truly proteiform malarial infections, we find complex nervous symptoms which cannot be easily referred to any known type of nervous disease. Thus Chiarini observed amaurosis and optic neuritis after a malarial attack in a man of thirty-four years, whose father died in a lunatic asylum, who had previously suffered from convulsions, and who was a drinker. A day of apyrexia intervened, and was followed by a quotidian fever for four days; during this attack and also in the period of apyrexia there were grave nervous symptoms, such as weakness and paralysis of the lower extremities, stupor, conjugate deviation of the eyes to the right, rectal and vesical paralysis, bulbar symptoms, and analgesia of the lower limbs and of the lower half of the body. On the fourth day the temperature rose to 41.5° C. (106.7° F.), and death occurred during coma. At the autopsy were found estivoautumnal parasites, a few in sporulation and a moderate number approaching sporulation, accumulated in the capillaries of the brain. In the eye was found an infiltration of leucocytes in the optic nerve, in the papilla, and in the surrounding retina, and in addition there were numerous leucocytes, with several melaniferous phagocytes.

The nervous symptoms in the cases so far recorded, to which further experience will probably make additions, were developed during either a febrile or an afebrile malarial infection. While some of them, as we have seen, disappeared rapidly, the infection being conquered, others were protracted for days and even for weeks. We shall return to this subject when treating of the morbid sequelæ.

Having described the chief forms of those pernicious fevers which might be called the cerebrospinal, we will proceed to the other varieties.

Choleraic Pernicious Fever.—Gastrointestinal disturbances are frequent in malarial infections; but only exceptionally do they attain such severity as to threaten life, as they do in choleraic pernicious fever.

The attack once ushered in, we have vomiting, abdominal pains, and frequent and copious diarrhœa. The stools are watery and stained with blood or bile and in either case rich in flakes of mucus; sometimes fæces, which are rice-water-like and abundant in the beginning, become scanty and mucosanguinolent later. A microscopic examination of the dejecta shows swollen and vacuolated leucocytes, phagocytes with red blood corpuscles, and sometimes red blood corpuscles containing annular and motile malarial parasites, and numerous microorganisms. The symptoms of the attack are so like those of cholera that in times of cholera epidemics an examination of the blood will be indispensable to the diagnosis of pernicious malaria; yet the presence of the malarial parasites does not exclude the possibility of choleraic infection, as was shown by a case observed in Rome in which the two infections existed in the same patient. At the acme of the attack, the patient is in much distress; there is a sense of oppression, his face is pale or cyanotic, the eyes are sunken, the skin is cold and covered with clammy perspiration, even if the rectal and the axillary temperature are febrile; the pulse is rapid, small, and thready, the tongue dry, the voice weak or lost; hiccough, severe thirst, and painful cramps in the lower limbs torture the patient; the urine is scanty or suppressed. Death may occur during this stage by a gradual aggravation of the symptoms followed by collapse, or it may come during full preservation of the intellectual faculties, or in delirium, in prostration, or in true coma. The most frequent result, however, is that the patient comes out of this state in a much improved condition and goes on rapidly towards recovery; the algidity diminishes from the centre towards the periphery, the diarrhœa ceases, the cyanosis disappears, and the distress gives way to calm followed by a long sleep from which the patient awakes refreshed and restored. An attack of pernicious cholera may occur in the first invasion of a fever or during a relapse.

We have already stated that in this disease the copious watery dejections are sometimes followed by rather scanty mucosanguinolent discharges of a dysenteric appearance, and that this change in the dejecta may precede a fatal issue. Cases of pernicious attacks have been described, however, in which a few diarrhœal discharges were followed by frequent mucosanguinolent dejections, abdominal pain, tenesmus, and vomiting of mucosanguinolent matter tinged with bile. In these pernicious forms the fever is high, the patient is in great distress and prostrated, with a small and rapid pulse, and the features are contracted. Algidity and cyanosis appear rather rarely. To febrile attacks presenting such marked symptoms Torti has given the name of *dysenteric pernicious fever*, and he has seen it occurring several times in the same patient until put a stop to by cinchona bark. Daullé (quoted by Kelsch and Kiener) states that in Madagascar he observed many cases of intermittent fever of dysenteric form. In these, during the cold stage, the patient was tormented by violent colic followed by mucosanguinolent dejections with tenesmus, and rapidly fell into grave prostration, with circulatory weakness and cold extremities; all of these symptoms disappeared with defervescence, which was accompanied by copious sweating.

Algid Pernicious Fever.—We have already noted that in choleraic pernicious fever algidity follows the gastrointestinal symptoms. But there is another pernicious type of extreme gravity, in which algidity with signs of collapse is the predominating symptom. The patient has a look of astonishment, the nose is sharp, the cheeks seem projecting, the lips and the extremities are cyanotic, the nails are livid, the pulse is frequent, small, and soft, becoming thready and finally imperceptible, the skin becomes cold from the periphery to the centre, icy cold, and is often covered with a cold clammy sweat. The respiration is frequently labored and interrupted, the breath is cold, and there is a raging thirst. Consciousness persists, but the patient seems not to realize the gravity of his condition, and answers slowly with a weak and tremulous voice. The algidity and the other symptoms may last several hours, rarely more. Death preceded by the tracheal râle is the most frequent result.

Thayer describes a very characteristic case of algid pernicious fever. The patient applied for admission to the hospital at eleven o'clock in the morning; the examining physician at once recognized that he was in a grave condition; he was cyanotic and prostrated, and his pulse was imperceptible. The blood was found to contain numerous estivoautumnal parasites. Death occurred two hours later, in spite of injections of quinine and of stimulants. A very similar case came under our own observation. The patient was a young

man of twenty-five years, who applied for admission to the hospital on August 11th, complaining of a quotidian intermittent fever. At the hospital, he had two attacks on the 12th and the 13th; at the afternoon visit of the 14th he was found to be in a condition of profound prostration, indifferent and as if resigned to die, with a cold and cyanotic skin, an imperceptible pulse, and an extremely weak voice. In spite of prompt measures he died during the night. In the blood during life, and in that of all the organs after death, numerous non-pigmented estivoautumnal parasites were found. Other similar cases have been reported by Sternberg, Laveran, and others. All writers agree in describing the profound impression made upon the physician by the aspect of an algid pernicious fever, and by the frequency of a fatal issue, in spite of specific and stimulating treatment. We have seen cases of algid fever with diarrhœas and dysenteric discharges, but these symptoms were so slight as not in the least to explain the algidity, the cyanosis, and the collapse as the results of inspissation of the blood, which in choleraic pernicious fever is the effect of the enormous loss of water from the digestive tract.

Diaphoretic Pernicious Fever.—In this deceptively insidious form, the culminating symptom is the profuse perspiration which comes on after the attack has lasted some time. From the patient's account we might think that the attack was terminating in the sweating stage, were it not that the sweat becomes more and more copious, the pulse small and rapid, the respiration labored, and the extremities cold. The collapse is most grave, but the patient's mind remains clear, and, as Torti says, who himself suffered from this disease, "sentit se paulatim mori."

Cardialgic Pernicious Fever.—During the attack a sharp pain occurs in the epigastric region, and this is often accompanied by frequent vomiting that becomes sanguineous. The patient is depressed and groans in anguish, his features are drawn into an expression of painful anxiety, the abdomen is retracted and painful, the tongue red and dry, the pulse thready, and the extremities are cold. Laveran has described a case of this kind which ended in recovery, but he cites Colin and Haspel, who hold cardialgic pernicious fever to be one of the most dangerous forms.

The varieties of pernicious fevers described above after the cerebrospinal were grouped together by Kelsch and Kiener, following the example of Dutrouleau, under the name of *algid fevers*, because the symptom common to all is that of algidity. These writers, moreover, appear not to admit the existence of an algid pernicious fever properly so called; that is to say, one in which the collapse, the most prominent characteristic of which is algidity, is the primary symp-

tom, and not the secondary as in the choleraic, the cardialgic, and the diaphoretic. Now, although it is true that algidity is observed more or less in all these pernicious fevers, this offers no valid argument against the existence of a genuine algid fever. Examples of this variety observed by us and by others oblige us to admit its existence without question.

Among the algid pernicious fevers, some writers place *syncopal pernicious fever*, Torti describing it after the diaphoretic, in the group of the coagulative. Now, syncope may occur in the course of the disease, but Torti and others call truly syncopal the febrile attack in which fainting occurs frequently, and during which death may take place in syncope. Sternberg notes also that a grave and prolonged febrile attack may be followed by death from syncope, and quotes an observation of Fayrer describing how an English officer in Calcutta, being in the sweating stage of a prolonged attack, with much weakness and a depressed pulse, insisted upon getting up from his bed in despite of the physician's orders, and fell dead upon the floor. But, in the face of this and of other cases of sudden death from syncope, we cannot help asking whether the event was due to malaria alone, or to some preëxisting cardiac lesion, to alcoholism, or to some idiosyncrasy.

In some cases the symptom of the greatest gravity is related to the respiratory organs, as in *pneumonic pernicious fever*. During the attack, the patient has dyspnœa and cough, with a sanguinolent expectoration. Physical examination shows that in a portion of the thorax, posteriorly, there is an area of slight dulness, with fine moist râles, and sometimes even bronchial breathing. These signs disappear with the cessation of the febrile attack, but may reappear in a succeeding one. This variety was described by Baccelli, Hertz, and then by other physicians (Mariotti, Gui, Ciarrocchi, and others).

Hertz has also described a case of *intermittent pleuritic pernicious fever*, in which there were sharp pricking pains, a dry cough, and marked friction sounds. All of the symptoms disappeared with the ending of the febrile attack.

Hemorrhagic Pernicious Fever.—In the estivoautumnal fevers we not infrequently find punctiform hemorrhages in the skin, the mucous membranes, the retina, and the brain, and these may be manifested in all the pernicious fevers. But there is one variety in which the symptom of the greatest gravity and danger consists in these hemorrhages. They are seen not only in the skin, which may be covered with them, but in the mucosa of the nose, the bronchi, the intestines, the stomach, and the genital organs, and they may be so abundant as to cause acute grave anæmia in a few hours, whence arise loss of

strength, a thready pulse, dulness of the sensorium, delirium, and convulsions. At the end of the attack the hemorrhages also cease, but the consequences of their occurrence may be such that recourse to all the known means of treating ordinary acute anæmia from hemorrhage may be necessary to avert a catastrophe. We must add that the anæmia secondary to this form of pernicious fever is of considerable duration, and is sometimes rebellious for a long period to treatment. Some writers subdivide the hemorrhagic pernicious fevers into various forms, giving each the name of the predominant hemorrhage; as scorbutic, epistactic, hæmoptysic, hæmatemetic, enterorrhagic, metrorrhagic, etc. Were call to mind the case of a robust man of thirty-two years, who was taken to the hospital of Santo Spirito from the Porta Angelica near it, suffering from dyspnœa and fever, and expectorating bright frothy blood. The history given was that of fever of a quotidian type which had lasted several days, the labored breathing and the bloody sputum having appeared a few hours previous to his admission to the hospital. Injections of quinine were given and the symptoms were treated, but the patient died in a few hours with symptoms of asphyxia. At the autopsy both lungs were found to be the seat of numerous recent hemorrhagic infarcts, a few of them lobar; there were also melanæmia and melanosis of the spleen, the liver, and the bone marrow. There were no lesions of the heart or of the large thoracic vessels.

Pernicious Fever with a Scarlatiniform Exanthem.—It is well known that during a malarial attack certain cutaneous eruptions, as herpes and especially urticaria, are apt to appear, but they are of no special significance, and rapidly subside. The same cannot be said of the diffuse scarlatiniform rash in cases of grave malaria. This form of pernicious fever was observed by the earlier physicians. Morton noted grave fevers *cum efflorescentia febrem scarlatinam simulante.* Bastianelli and Bignami describe a case of malarial infection in which there was a rash like that of diffuse scarlatina covering the whole body, with erythema of the fauces. The erythema returned after desquamation in large scales had already lasted for three days. During the eruption an examination of the blood showed the presence of numerous estivoautumnal parasites. A typhoid condition followed the second eruption, accompanied by grave icterus and diarrhœa, the patient becoming progressively more anæmic. The parasites in the blood gradually diminished in number. At the autopsy, in addition to the lesions of malaria, there was found a necrotic zone in the liver with resulting emboli of hepatic cells in the suprahepatic veins. This relapsing scarlatiniform erythema in malarial infection recalls those rare cases of the same eruption which have been described as

occurring after certain infections, especially acute articular rheumatism, and also typhoid fever and pneumonia, cases of which we have recently had occasion to observe.

The pernicious infection, of which we have just described the principal forms, is designated pernicious fever because the fever, which is as a rule high, persists in the greater number of cases, and is sometimes of great assistance in the differential diagnosis. But in some exceptional cases the fever may be absent, even when a large number of parasites is found in the blood; we then have the afebrile pernicious syndrome, to which the earlier writers gave the name of concealed or larval pernicious fever, and of which we have given some examples.

Towards the end of an autumn which has been characterized by dampness and sirocco winds, many cases of pernicious malaria may be observed, the termination of which is always fatal even after two days of the administration of large doses of quinine. The course of the disease in these cases is apyretic, or else accompanied by slight elevations of temperature, with symptoms of collapse, and sometimes a rapidly occurring terminal coma. Even in the cases which have been treated with quinine, enormous numbers of parasites are found at the autopsy.

Now why is it that when all the conditions for the production of fever are present, the fever does not appear? The question is not an easy one to answer, any more than it is easy to say why in some cases the fever continues for several days even when the parasites gradually disappear. The new elements which come in to destroy the link between the exciting causes of the fever and the fever itself are as yet unknown to us. But are we any wiser as to the reasons for the same kind of thing in other infections, as, for instance, typhoid fever?

An *examination of the blood* in the pernicious intermittent fevers shows the presence of many estivoautumnal parasites, all, or nearly all of them, in the same stage of existence, so that we may call them one generation of parasites; this is also shown by the post-mortem researches made in the case of fatal pernicious fevers, in which in any organ, such as the capillaries of the brain, we find all the parasites to be either non-pigmented, or without granules, or with blocks of pigment, or in segmentation. But in pernicious malaria marked by the presence of subcontinuous fever, the blood contents are more complex; that is to say, we find parasites in various stages of development, so that we may assert that there are several generations of them. This is confirmed at the autopsy, at which parasites in every degree of development are found—in the brain capillaries, for ex-

ample. In the pernicious infections, an examination of the blood always gives positive results, except in a few cases which we shall mention presently; and indeed in this disease we often obtain blood preparations which are truly surprising for the number of parasites which they contain.

Causes of Perniciousness in Malaria.

We now come to a description of the researches which have been made with a view of ascertaining the reasons for the pernicious character of malarial affections considered from the point of view of the pathogenic agent. The parasitic data in pernicious infections are (1) the presence of estivoautumnal parasites, and (2) their abundance.

Every one is now agreed as to the first. And yet not all the fevers produced by these parasites are pernicious, nor even grave; some are mild, and some resolve spontaneously. But this does not invalidate the previous statement.

As to the second, the abundance of the parasites, it will be necessary to consider the subject a little more at length. All those who have had occasion to study cases of pernicious fever since the discovery of the parasite have perceived this abundance of the parasites either in blood taken from the finger or in that collected at autopsy. Bignami, in his work upon the pathological anatomy of pernicious fevers, has called special attention to the fact that the contradiction found so often during life between the number of the parasites and the gravity of the disease, and also the degree of anæmia in the majority of cases, disappears when an autopsy allows of an examination of all the organs. Further researches have confirmed his statement, and have justified the assertion made by Bignami and Bastianelli, which is that "in pernicious fevers, if we examine not only blood taken from the finger, but also that from the vessels of the various organs, the chief fact made evident is that, although their distribution may vary in certain cases, the number of the parasites is always very great." We have already spoken of the varied distribution of the parasites whence proceeds the variety in *parasitic localization*. Now, while in cases in which the distribution of parasites is fairly even their abundance will be shown in blood taken from the finger, the same does not obtain when there is definite localization. In the latter event there may be but few parasites in finger blood, and yet we may be able to judge of the gravity of the disease and to infer the hidden abundance of the parasites, because the scantiness of their number is often compensated for by the appearance of the parasitic bodies as regards the stage of their existence, it being a common occurrence to find those containing blocks of central pigment

the presence of which, as experience has taught us, is the sure indication of the accumulation of vast numbers of parasites in the viscera.

We must, however, confess that there are cases in which the disproportion between the number of parasites found in blood drawn from the finger and those shown by microscopic examination to be in the organs is very considerable. This is found in some pernicious fevers of protracted course, which have been subjected to regular treatment, in which the parasites in the blood diminish little by little, but the cerebral symptoms meanwhile persist, a fact accounted for at the autopsy by the great accumulation of parasites in the capillaries of the brain and the meninges. This also occurs in certain cases of pernicious fevers which have been unmodified by treatment, that is to say, in patients who die a few hours after having been admitted to the hospital. Bastianelli and we have observed cases of pernicious fever of this variety in which a first examination of blood from the finger, and a subsequent one of that from the peripheral veins after death showed the very smallest possible number of parasites. At the autopsy, while few parasites and very slight melanosis were found in the spleen, the bone marrow, and the gastrointestinal mucous membrane, yet melanosis was perceptible macroscopically in the brain, and the capillaries of the cortex and the meninges were found to be overflowing with parasite-containing red corpuscles, the parasites being all in one stage (with blocks of pigment) or in various stages of existence. It is to be observed that the cases with slight spleno-hepatico-medullary melanosis were those in which a lethal pernicious fever supervened in one of the first attacks of a primary infection; and that the cases in which the disproportion was great between the number of parasites found in the finger blood and in the organs in which they had become localized were, according to our observations, those of cerebral localization, that is to say, those in which an enormous mass of parasites had accumulated in the vessels of the brain and of the meninges.

We must therefore conclude that by the side of cases in which a clear diagnosis and prognosis can be given from a study of the parasites in blood taken from the finger, there are others in which this procedure as well as the study of blood from the spleen will be of no assistance. The first cases are, however, the most frequent, and in fact the rule, the others being the exceptions.

Some further remarks upon the number of the parasites found in pernicious infections will be in place.

There are cases of long duration in which, under the influence of quinine, the parasites gradually diminish and disappear, and yet the pernicious symptoms persist. Some such cases have been observed

by ourselves, by Celli, and by Bastianelli. The patients were brought to the hospital with pernicious symptoms, and an examination of the blood showed an abundant, sometimes an excessive, number of parasites. The patients recovered promptly and perfectly under the use of the specific or other remedies; after twenty-four hours the parasites were already diminished in number, their diminution continuing until the third or fourth day when they entirely disappeared. The fever, however, continued, or remitted, or became intermittent, and then rose again, and often reached a tolerably high degree; the pernicious symptoms remained; coma was followed by delirium, the anæmia became more grave, and a fatal issue occurred even in hyperpyrexia. The autopsy showed great melanosis of the spleen, the liver, and the bone marrow; but the parasites either were present in small amount or were altogether absent, except for a few crescent bodies in the spleen and the bone marrow.

Death may therefore occur in pernicious infections even after a notable diminution or total disappearance of the parasites. The same thing sometimes happens in other infections, as in pneumonia, diphtheria, typhoid fever, etc. Thus the diminution and disappearance of the parasites from the finger blood does not always warrant a favorable prognosis, nor, as we have seen, does it always signify that the parasites from the peripheral circulation have accumulated in the internal organs. In the class of cases discussed, although the parasites have been destroyed by specific treatment, the alterations due to their action persist: grave acute anæmia, more severe than that secondary to hemorrhages, because in malaria there remains a detritus of red corpuscles, pigment, dead parasites, and phagocytes, to which is due perhaps the production of toxic substances not influenced by quinine; punctiform hemorrhages which are sometimes scattered throughout the white substance of the brain, the crura cerebri, and the spinal medullary substance; necrosis of the liver and kidneys, and alterations in the endothelial layer of the capillaries of the brain, whence the retrogressive changes in the nerve cells.

An exception—really one of appearance only—to what has previously been stated in regard to the number of the parasites in cases of pernicious infections is found in a series of cases of clinically grave forms of the disease (comatose, convulsive, delirious, or mixed) in which from the beginning to the end of the disease we find very few parasites, even at the autopsy no signs being found of a progressive infection due to a large number of parasites—especially melanosis. These are rare cases, which as a rule occur at the beginning of the malarial season, in the month of July, or even later if the heat continues, or if after a slight cooling of the temperature in August great

heat comes on again at the end of that month or in the early days of September; but this is even more rare. The patients are young, robust men, laborers in the country, occupied in threshing grain or in preparing the earth for sowing, or else persons whose occupation obliges them to remain for many hours of the day in the country in the sun, or sailors stopping for a little while in malarial regions, or fishermen. When the patients come under medical observation, they are already in a grave condition of prostration, or comatose, or delirious, and have a high fever, 40°–41° C. (104°–105.8° F.) which is rarely intermittent, more often continued. Convulsions and coma are seen in the cases with a rapid course; delirium which is sometimes furious in its nature, in those of a more protracted course (three to five days). The spleen is enlarged. An examination of the blood shows few parasites. At the autopsy we find severe cerebral hyperæmia, an acute enlargement of the spleen, which is slightly melanotic, and parenchymatous changes in the kidneys. The parasites are scanty, particularly so in the brain, the bone marrow, and the spleen; and the melanosis of these organs and of the liver is slight.

In persons who have been energetically treated with quinine, we do not find any parasites, but there is slight melanosis, so situated as to demonstrate a recently exhausted malarial infection.

In a case which occurred in the middle of July, the patient was a young sailor of the most robust build, who came from Genoa and stopped for five or six days at Fiumicino. After leaving this place, at Rome by the Tiber, he was taken with a severe headache, high fever, and then a raging delirium. The fever fell one day before his death, but collapse occurred, in which the patient muttered words whose sense could not be understood. At the time of his admission to the hospital there were a few non-pigmented parasites, and very few pigmented leucocytes. Death occurred on the third day, when the examination of the blood gave negative results. At the autopsy were found very slight melanosis and no parasites, not even those of the crescent stage. Now what is the explanation of these cases, which cannot be accounted for by the knowledge which we have gained in regard to the pathogenesis of pernicious infections?

Many writers get out of the difficulty in an easy way by saying that these grave infections are due to great virulence on the part of the estivoautumnal parasites, which compensates for the scantiness of their numbers. To hold that the lethal gravity of these cases is the result of a few parasites, as few as are seen in the mild infections, but extraordinarily toxic in their nature, is a mere hypothesis. Bastianelli and ourselves have long discussed this point, and it has become our opinion that the cases recorded were cases of sunstroke

complicated by malaria. The following reasons lead to this conclusion: (1) The persons affected by the disease work in the open country exposed to the rays of a fierce summer sun, and they work hard, in the midst of poverty and insufficient nourishment; (2) cases of sunstroke do occur, even though rarely, in the Roman Campagna; (3) the clinical signs of these cases correspond to those proper to insolation; (4) the anatomico-pathological data correspond equally well to those of insolation, which is the result chiefly of cerebral and pulmonary hyperæmia; (5) cases of sunstroke complicated by malaria have been described as occurring in hot countries, as, for example, by Kelsch and Kiener in Algeria.

During the summer in Rome we sometimes have occasion to observe fevers which are usually continuous, and last six, eight, or nine days, accompanied by grave nervous symptoms, and tending to a fatal issue. Physicians usually class these with the subcontinuous malarial fevers. Nervous symptoms appear in the very beginning of the disease, and we note an incoherent delirium with much agitation, or unconsciousness, or coma up to the end. The spleen is only slightly enlarged, the abdomen is retracted, the urine may be albuminous. No malarial parasites nor pigmented leucocytes are found. Quinine is of no use, even in large doses. At the autopsy we find cerebral hyperæmia, slight enlargement of the spleen, a flaccid dilated heart, and cloudy swelling of the liver and kidneys, but no lesions of malarial origin. The subjects affected are countrymen, fishermen, etc., men who work in the sun. Are the cases examples of sunstroke or of some other disease of unknown etiology analogous to the "ardent continued fever" of hot countries described by Murchison? Further researches are needed to throw light on the matter.

But, while further researches are necessary to an elucidation of the matter, we may after this digression repeat what we maintained at the beginning, to wit, that in pernicious infections, all things considered, the number of parasites present is considerable.

It is superfluous to add that the parasites may be all of one generation, or of several, as was first observed by Bignami, who regarded this as one of the most important factors of the perniciousness; only, however, for the estivoautumnal parasites, because two or three generations of quartan or tertian parasites may be found in the blood of the same patient without the fever assuming a pernicious character.

Two biological properties are quickly recognized in the estivoautumnal parasite, which distinguish them from other malarial parasites, and which reveal the malignity causing the pernicious course of many of the cases of infection; these are the greater proliferative activity and the higher toxicity.

The greater activity in multiplication of the parasites is shown by the enormous parasitic invasion found in the majority of cases of pernicious fever, revealed not only by a microscopic examination of the viscera, but often by that of blood taken from the finger, in which at the acme of the attack are found numerous endoglobular amœbæ, more than one being frequently seen in the same red corpuscle. In spite of this great proliferative activity, it is rare to find bodies multiplying in the peripheral blood, and when we do find such the case is certain to be one of grave infection. This is the reason of the difficulty experienced in ascertaining the life cycle of this variety of parasite. Another proof of the greater capacity for multiplication possessed by these malarial parasites has been given in what was said in relation to the experimental researches made upon the period of incubation in malarial fevers. From these studies we learn that the shortest period of incubation is found in the fevers caused by the estivoautumnal parasite; and since the period of incubation means nothing more than the time necessary for the parasites, by multiplication, to attain sufficient numbers to produce the fever, it may readily be deduced from the brief incubation stage that not only is the life cycle of the estivoautumnal variety of parasite more rapid than that of other forms (as is seen in quotidian fever), but that the capacity for multiplication of this species is greater than that of any other having the same duration of life. Finally, it is to be noted that the number of spores of the estivoautumnal parasites are often as many as ten, fourteen, or twenty, and may even reach twenty-two, twenty-five, or thirty.

Our knowledge of the *toxic power* of the malarial parasite is small, and further investigations are necessary to an understanding of the toxins of malaria as exact as those which we possess as to the toxins of diphtheria and certain other diseases. We may infer the existence of this toxic power from the fever, and from several retrogressive changes found in the bodies of patients who have died of pernicious fever. As to the fever, it is admitted that during the process of segmentation a pyretogenous toxin is set free. But the question of the derivation of this toxin, whether it comes from the parasite or from the detritus of the destroyed red corpuscles, still remains unanswered. The chief of the retrogressive changes is the brassy change in the red blood corpuscles. This alteration, which up to a certain point distinguishes the estivoautumnal amœba from those of the quartan or the ordinary tertian, would tend to prove that even during the development of endoglobular life a substance or substances are produced which so profoundly change the red corpuscles as to make them lose their fundamental characteristic of elasticity, whence the difficulty

they experience in getting through the lumen of the capillaries. Other proofs of the greater toxicity of the amœba are given by the extensive necrosis of the renal epithelium, especially in the convoluted tubules, a necrosis which is not secondary only to alterations in the blood-vessels produced by the parasites; and by some extensive necroses of the liver epithelium, and by the malarial hæmoglobinuria, which has so far been found only in estivoautumnal infections. Moreover, it may be said that to the greater toxicity of these parasites is due the gravity of the disease which they produce. Finally, another sign of the malignity of estivoautumnal parasites is given by the greater resistance which they oppose to the salts of quinine. Cases have occurred, as we shall see when speaking of the action of quinine in this group of fevers, in which the specific remedy, given in large doses by the mouth or by hypodermic injections, did not avert a pernicious attack, the diagnosis of which was established by examination of the blood, nor a second attack, nor a fatal issue. In these cases, even after the prompt and energetic administration of quinine, the parasitic cycle goes on to completion until the death of the patient occurs.

Other factors of the perniciousness, besides those due to the parasitic agent considered both as to its quality and its quantity, may be found, as in all other diseases, in such *individual conditions* as diminish resistance to the infection. It is well known, for example, as stated by Colin and others, that the grave and the pernicious continued malarial fevers more often affect those who have been living but a short time in malarial regions than they do those native to the place, that the grave forms are less frequent in patients with chronic malaria, that children are more frequently affected by eclamptic pernicious fever, and old people by the paralytic form, etc. A few important data in this connection have been revealed by pathological anatomy; for instance, several cases of pernicious fever in which we made autopsies, occurred in those affected by arteriosclerosis, and also in individuals in a more or less advanced stage of interstitial nephritis, or with arteriosclerotic lesions of the heart. If we bear in mind the acute dilatation of the heart which occurs during pernicious fevers, and the rapid aggravation of the disease which follows the weakening of the myocardium, we can understand how the lesions mentioned above, which so easily cause weakness of the heart, have such an important bearing upon the result of the grave malarial infections. Alcoholism, overwork, insufficient nourishment are all factors in the gravity of malaria. Baccelli attributes much importance to individual conditions in the genesis of perniciousness; he even holds that the complicated pernicious fevers are entirely due to a

predisposition of the organs and systems from which the pernicious symptoms arise; while subcontinuous pernicious fevers he holds to be due to the intensity of the cause acting upon the organism of the patient. From what we have said in regard to the parasitic data, we find that the parasites of the complicated and those of the subcontinuous form are the same; that is to say, they are the same in the various stages of their development in several generations, in fact.

Pathogenesis of the Pernicious Symptoms.

The facts revealed by pathological anatomy and by a study of the biological properties of the parasites in grave infections enable us to make further researches with a view of ascertaining whether they are sufficient to account for the symptoms observed in these infections, and especially for those in the complicated form, in explanation of which many ingenious theories were advanced by the earlier writers. Among the most frequent symptoms of pernicious fevers, and those which attracted the chief attention of observers, were the cerebral, more especially the comatose.

Frerichs described accumulations of pigment in the cerebral capillaries causing their occlusion, and hence capillary apoplexy; as to the causal relation between these lesions and the clinical cerebral symptoms, he expressed himself with much reserve, because he saw so many cases of melanosis of the brain unaccompanied by cerebral symptoms, and so many cases of cerebral symptoms without melanosis. The latter condition he found in six cases out of twenty-eight. Therefore Frerichs held that other factors besides melanosis were essential to the production of pernicious cerebral symptoms. He suggested that possibly a study of the chemical products set free by the destruction of the red blood corpuscles might lead to a comprehension of the phenomena.

Laveran, having recognized the parasitic nature of the pigmented bodies, attributed the cerebral symptoms to their accumulation in the cerebral capillaries where they form true *parasitic thrombi*. This theory, according to Laveran, is in harmony with certain facts observed in the course of pernicious fevers, among others the occasionally rapid disappearance of the cerebral symptoms and the marvellous action of quinine.

But later researches permit of a different conception of the origin of the cerebral symptoms, and we now have the theory of *endoglobular parasitism*, and the consequent alterations in the red blood corpuscles.

Through these alterations the infected corpuscles lose their elas-

ticity, and their surface becoming irregular offers a greater resistance than normal to the circulation; this causes their accumulation at the periphery of the venous circulation, and slowly and with difficulty they traverse the narrow capillary territory in which the degenerative changes of the endothelium secondary to the defective circulation become an added cause of serious slowing of the blood current (a slowing which is a useful and perhaps necessary condition of the multiplication of the parasites) until stasis of the corpuscles occurs. These circulatory changes are shown in the anatomico-pathological examination, when we can see the increase in the endocranial tension, and intense cerebral and meningeal hyperæmia through the accumulation in the cerebral capillaries of amœba-infected red corpuscles, while many normal red corpuscles are found in the larger veins. The fact that in capillary hemorrhages the escaped corpuscles are nearly all normal, while the cerebral capillaries from which they originally came contain many parasitic corpuscles, is a further proof of the changes undergone by the latter. Only exceptionally do we find in the cerebral capillaries accumulations of blocks of free pigment (pigmentary thrombosis), or accumulations of melaniferous leucocytes (phagocytic thrombosis), or finally accumulations of free parasites, especially free spores (parasitic thrombosis); for this reason these facts are of less importance than the accumulation of the parasite-infected red blood corpuscles.

Now it is unlikely that the slow circulation in the cerebral capillaries of corpuscles so altered, and certainly incapable of taking up oxygen or of taking it up in normal amount, should not be a cause of functional and nutritional changes in the nervous centres, through deficiency of nutritive material and of oxygen. That retrogressive changes in the nerve cells of the cerebrum and the crura cerebri can occur in pernicious malarial infections has been demonstrated by recent researches, as we have already seen. This being the case, we are quite entitled to hold that in the anatomico-pathological data there are to be found satisfactory reasons for the cerebral symptoms of pernicious fever, such as the unconsciousness, coma, convulsions, delirium, etc., as well as for the aphasia, hemiplegia, and bulbar paralysis. The persistence of some of these nervous disturbances after the attacks have disappeared is the consequence of the delicate changes in the nerve cells, in which restoration gradually occurs by the return of normal metabolism and the reconstitution of the blood.

The results of recent investigations enable us to answer the objections raised against this theory which explains the theory of pernicious infections. In judging of the cases in which death

occurs in coma, and in which at the autopsy we find cerebral hyperæmia without melanosis, we must bear in mind the fact that sometimes the cerebral vessels are full of red corpuscles all or nearly all of which contain non-pigmented amœbæ, the stage of accumulation of the sporulating bodies having already been passed. In the second place, in those cases in which death occurred in coma, and cerebral melanosis is found at the autopsy, we must not omit to note the degree of the melanosis and must try to ascertain whether it is found along the blood-vessel walls as a residuum of parasitic invasions, or is dependent upon the presence of pigmented parasites within the vessels. Moreover, we must not forget to take into account the rapidity with which the parasitic invasion takes place; the effect upon the cerebral functions must certainly differ according to whether the invasion of red corpuscles containing mature parasites, such as occurs in the beginning of the attack, is accomplished quickly, causing fatal coma, or slowly and by degrees as we have reason to think that it is in some cases.

The same circulatory changes that are found in the capillaries of the brain occur also in other organs and systems, and form the anatomico-pathological basis for other forms of pernicious attack. Thus choleraic pernicious fever is well accounted for by the alterations of the mucous membrane of the stomach and intestines secondary to the accumulation of parasite-infested red corpuscles and even of phagocytes in the capillaries of those organs, occasioning an abundant transudation, which is sometimes bloody, and is the principal symptom of the affection.

But other pernicious symptoms, such as we see in the algid, cardialgic, and hemorrhagic, and other forms, undoubtedly arise from complex causes, and have up to the present time escaped any interpretation founded upon a convincingly sufficient number of observations, unless we can content ourselves with the explanation that they are the result of an intoxication—which, according to some writers, accounts also for the cerebral symptoms. But this chemical theory as to the symptoms of cerebral pernicious fever seems to us to be superfluous in view of the many data obtained from an anatomico-pathological examination, and also from a clinical standpoint. The vascular changes in the brain and in the pia mater have already been described. From clinical reports given we also recall the rapid disappearance and the rapid reappearance of the cerebral symptoms, as was observed in a case of two pernicious attacks following each other and separated by a more or less long period of apyrexia. This occurrence, which can be readily explained by the circulatory changes in the brain, it would be difficult to believe the result of poisoning. All that we have learned

in regard to the alteration of the red blood corpuscles in estivo-autumnal infections, and the variation in the number of the parasites circulating in the peripheral blood, in their relation to the symptoms of the attacks, has long inclined us to hold that the greatest accumulation in the cerebral capillaries and in the capillaries of other organs, as the intestinal mucosa for instance, occurs during the sporulating phase of the parasites. The spores once detached, the red blood corpuscles disintegrate and leave a free passage to new red corpuscles, the carriers of oxygen, whence the gradual diminution and cessation of the cerebral symptoms, such as coma. In some cases this persists in spite of the diminution and disappearance of the parasites even from the cerebral capillaries. The explanation is not hard to find in the changes in the nerve cells, as well as in those in the endothelium, forming an impediment to the circulation of the red corpuscles which are normal or only slightly altered as when they contain young parasites. Sometimes the cause is found in the numerous punctiform hemorrhages, though we would not absolutely exclude the influence of certain of the toxic products, as we have already said elsewhere.

Tropical Malaria.

The tropical malarial fevers properly so called do not differ in their essential characteristics from our estivoautumnal fevers; this may be demonstrated by clinical as well as by bacteriological data.

During the last few years we have gathered together from the writings of tropical physicians, the English and the German especially, a large number of records of observations, which have enabled us to compare the clinical and parasitic data of tropical malaria with those of European malaria. From these studies we draw the deductions that (1) tropical fevers do not clinically differ in their essential characteristics from the grave forms seen by us; and (2) in the great majority of cases in the tropics the parasites found in the blood do not differ essentially from those which we have described as estivo-autumnal parasites.

Varieties.—In fact from the reports given by the various writers, which we shall summarize presently, it appears that in tropical countries every variety of malaria found in Europe may occur, although with variable frequency. *Quartan infection* is rare, and in some tropical countries, altogether lacking; *tertian infection*, on the other hand, is frequent and is always found in connection with the third variety, *estivoautumnal infection*. The latter, however, assumes the ascendant in frequency and gravity, so that when "tropical malaria" is men-

tioned without other qualification we understand the term as meaning estivoautumnal infection or even, according to the very recent researches of R. Koch, estivoautumnal tertian fever.

We shall not concern ourselves with the quartan and the ordinary tertian as observed in the tropics, because according to all writers, they do not differ clinically or in respect to the parasites from the same forms as seen by us at home. But a few brief references to the investigations made in regard to the third variety, *tropical malaria* properly so called, will be in place, because they are so many documentary proofs of the statements previously made.

In a former section we quoted several authorities, as Gueguen, Schellong, and others, in their descriptions and classifications of tropical fevers according to the symptoms and the temperature curve, and we stated that in all probability some of the fevers described by them as malarial did not belong to this class of infection at all. But in regard to the question now before us, our only interest lies in such observations as are accompanied by accurate investigations in regard to the malarial parasite, so described that we are able to recognize the variety of parasite referred to. In the first place we note that A. Monti has found the same parasitic forms that are seen in our grave fevers, in some cases of infection in Panama and other parts of Colombia, and in Venezuela. Among the researches made in America the first are perhaps those of G. Dock, pursued in Galveston in the subtropics. The majority of the cases studied by this writer came from the interior of Texas. From the descriptions which he gives, it is evident that he found the same parasites as those described by Laveran and by the Italian writers. In a later publication Dock gives the results of a hæmatological and anatomico-pathological study of a case of pernicious fever in which were found the estivoautumnal parasites. These same parasites have also been found in researches made in Mexico, Brazil, etc.

In regard to malaria in the tropical regions of Africa, we have reports from Thin, Duggan, Marchoux, R. Koch, A. Plehn, F. Plehn, and others. G. Thin describes a case of comatose pernicious fever observed on the West coast of Africa. As the author says, the results of the anatomico-pathological examination harmonize in all essentials with those obtained by Bignami. He describes in the brain an accumulation of parasites (as is usually seen in comatose pernicious fevers) in every stage of development, which from the account given appear to be identical with our estivoautumnal parasites.

C. W. Duggan, who has studied the fevers of Sierra Leone, has found the parasite in every case. In four hundred cases of fever he saw only one of ordinary tertian; in all the others there were the

small estivoautumnal parasites, and in the greater number of cases crescent forms as well. He never observed fission forms in the peripheral blood, but in cases of pernicious fever he found in the brain fission forms occupying not more than one-third of the red corpuscle and with but few spores. He never saw the variety of red corpuscles which we call "brassy," and this constitutes the only difference in the results obtained by this writer and by ourselves.

As to the clinical types, he says that in a newcomer the fevers of first invasion are usually of a quotidian remittent type, and for the most part are ushered in abruptly and often without a chill. The most frequent form of pernicious fever is, as with us, the comatose.

E. Marchoux conducted his investigations in the French colony at Senegal, which is situated between the twelfth and the sixteenth degrees of north latitude. Malaria is about the only serious disease found there. The parasites found by this writer are those of estivoautumnal fever, as evidenced by the descriptions given and the pictures subjoined. The writer describes fission forms with central pigment, which he found massed together in the capillaries of the viscera; he states, however, that the parasite completes its whole cycle of development without forming pigment. The fission forms have eight to twelve segments. The crescent bodies appear in the blood about the twelfth day. In three cases of comatose pernicious fever in which he made microscopical examinations, he found the same alterations that we are accustomed to see. Everything, therefore, leads us to believe what we stated above, that the parasite is the same as the one observed in Rome, and this view Marchoux himself shares. As to the clinical characters, he observes that the prevailing types are those with a tendency to become continuous; under treatment they become intermittent, then stop, and relapse after twelve to fourteen days. With the relapse, although of course not in every case, there is established a chronic condition of fever, which returns at somewhat regular intervals, with anæmia, large splenic tumor, enlarged liver, etc. Icterus is often present. The pernicious attack is always to be feared. A never absent symptom is albuminous urine, which rarely appears during the fever, but on the day or days following it. The most frequent complication is pulmonary congestion, and indeed many patients sent to the hospital for bronchopneumonia are afterwards, by an examination of the blood, found to be suffering from malaria. These fevers prevail during the whole of the rainy season, which lasts from July to October. From December on there are relapses only. During the dry season the country is healthy.

To the list of authorities we may add the name of Grawitz, who several years ago found the estivoautumnal parasites in the blood of

some soldiers who had caught the fever in Eastern Africa; A. Plehn, who found them in the hæmoglobinuria of Kamerun; and F. Plehn, whose descriptions give further weight to the arguments adduced.

As to the clinical forms of the fever in Kamerun, F. Plehn, in one hundred and thirteen temperature tracings in Europeans, found eight cases of tertian, twenty-nine regular quotidian, seventy-six irregular intermittent, and no quartan; he found no subintrant nor duplicate fevers, nor regular postponentes nor anteponentes. True continuous fevers, whose temperatures for more than forty-eight hours do not fall below 39° C. (102.2° F.), are, according to this writer, due to complications, chiefly pneumonia. The attacks may cease spontaneously, especially in the natives to whom quinine is not so indispensable as it is to Europeans. There is nothing of special interest in the clinical descriptions of the attacks. But it is a fact worthy of attention that in Kamerun notable enlargement of the spleen is rare even in those who have had many attacks of the fever. A true chronic infection is not often seen, possibly because only the most resistant persons remain in the colony. Patients are apt to complain more of pain in the liver than of that in the spleen. Albuminuria is rare; out of one hundred and ninety-eight cases it was found in only six. As to grave cases, those dangerous to life, in Kamerun as on the greater part of the West coast of tropical Africa, the hæmoglobinuric is almost the only form of practical importance. On the West coast, the other forms of pernicious malaria, described in other regions and seen by the writer on the East coast, have but small importance.

In Java, A. van der Scheer studied one hundred and five cases of malaria; in forty-two of them the parasites of the tertian and the quartan were found, in sixty-three the small plasmodia, and in two there was a mixed infection; thirty-one times the writer found crescent bodies. He noted that in grave fevers, if the examination was made during the sporulating stage, there might be no parasites at all found in blood from the finger, and only a few some hours later. These and other reports, which for the sake of brevity we omit, confirm the opinion that the parasites in these fevers behave as they do in our estivoautumnal forms. Only in Java it would appear that there is a greater preponderance of tertian and quartan forms than is usual in hot countries. As to the clinical types, the writer says that he has but rarely found pure quartan and tertian, but usually quotidian of tertian or quartan origin. The fevers caused by the small plasmodium are described as quotidian, remittent, subcontinuous, and pernicious; rarely tertian, more often quotidian, but for the most part with intervals of from twenty-four to forty-eight hours.

The same parasitic forms have been seen by various observers in the British Indies.

Among all these researches, the ones possessing the greatest special interest are those of R. Koch, which are of the utmost assistance to a right understanding of the tropical fevers. In German East Africa, Koch found chiefly two kinds of malaria; one the ordinary tertian, which occurs for the most part as a double tertian, and neither clinically nor parasitically differs from the ordinary tertian of our own climates. This tertian constitutes ten per cent. of the cases of malaria. In the remaining ninety per cent. is found the tropical fever properly so called. According to Koch's observations, the curve of this fever is not so irregular and multiform as physicians in the tropics have always believed, possibly because they have never seen the curve unmodified by treatment, but it is composed of attacks as typical and regular as those of our ordinary tertian. Only the curve of the attack has a somewhat diverse form, which corresponds perfectly to that of the estivoautumnal tertian described by us. The development of the parasites is as regular as that of the attacks, the two being in perfect correspondence. At the beginning of the attack there are small annular parasites about one-sixth the diameter of a red blood corpuscle; towards the end of the attack larger annular forms begin to appear; when the temperature has fallen, we find large annular forms, one side of the ring being thickened and falciform. The sporulating bodies of this parasite are not found in blood from the finger, but in that of the spleen; they are very like those of ordinary tertian, but are smaller. In spite of all treatment, this fever almost always relapses with regularity in from ten to fourteen days, sometimes after three or four weeks or more.

Hæmoglobinuric fever, which until now has always been considered as one of the gravest forms of tropical disease, is, according to Koch, not directly related to malaria; as a rule, he says, it is the result of quinine poisoning.

From the facts given above, and to which it seems to us superfluous to add further examples, it appears evident that, if we exclude from the list of tropical malarial infections a certain number of fevers formerly considered to be malarial but now shown by the negative results of an examination of the blood not to be such (as some "remittent continued," "typhomalarial" fevers, etc.), the tropical fevers do not differ in essentials from our estivoautumnal fevers. This may be asserted from both the parasitic and the clinical data.

As to the former, nearly all the observations reported show that in tropical countries two kinds of malaria predominate, those which we have called the *tertian infection* and the *estivoautumnal*, but the

second variety represents the febrile endemic characteristic of the region, *tropical fever* properly so called.

All the reports tend to prove that the parasites are the same as the ones observed by us. We may add that in recent years we have had occasion to examine the blood of several persons who had taken the fever in Abyssinia, and found in it the same estivoautumnal parasites. This was also the case in an examination made by Marchiafava of a traveller who became affected in the French Congo. The cycle of existence of the parasites, which could be followed in the peripheral blood, the accumulation of fission forms in the deeply seated organs, the structure of the parasites, etc., all corresponded in the most perfect way with the ones known to us.

Only F. Plehn a few years ago described in the fevers of Kamerun some parasitic forms which appeared to be incapable of staining in the dried preparations, and which did not become pigmented; clinically they produced grave and atypical fevers, uninfluenced by quinine, with but slight changes in the spleen and often accompanied by hæmoglobinuria. Fresh preparations were stained by methyl blue dissolved in serum. We may suppose that Plehn mistook alterations in the red blood corpuscles, which are easily shown by this method, for parasites. *A priori*, however, we find no difficulty in admitting the existence of a non-pigmented parasite of the red corpuscle which is stained with difficulty in dried preparations, for we have recently seen a parasite found by Dionisi in a bat which was most resistant to staining. But the descriptions given by Plehn are not sufficiently convincing to force us to the conclusion that the bodies he found were parasites.

Another slight difference between the parasites of tropical fevers and those of the estivoautumnal infections might be found in the lesser amount of pigmentation of the former. Marchoux, as we have already noted, describes as a matter of common occurrence the complete development of a parasitic cycle without the formation of pigment. The same fact has been noted by ourselves, although very exceptionally, and we have already given the reasons why we do not consider ourselves authorized as yet to recognize a parasite which never becomes pigmented as a species by itself pathogenic to man. In our opinion, we have not up to the present time a sufficient number of well-attested observations to justify such a conclusion. Apart from this question, the fact that there are slight variations in the amount of pigment found in the parasites of tropical malaria and of the malaria of our own countries cannot be held to constitute a real distinction between the two.

As to the clinical types, the majority of writers describe the tropi-

cal fevers just as our grave fevers used to be described by the greater number of physicians before our own observations were made. They speak of fevers with a protracted course and without the characteristic interruptions (continued fevers), of remittent fevers, and of intermittent irregular or quotidian fevers; the expression "irregular fevers" is the one most used. But the recent researches of Koch lead us to believe that the fever of the tropics is not so irregular and multiform as has been believed, but is regularly a tertian with prolonged attacks. Now it is easy to convince ourselves, by a comparison of Koch's temperature curves with our own, that this form of tertian, which is the typical tropical malarial fever constituting ninety per cent. of all the cases of malaria occurring on the East coast of Africa, is absolutely identical with our own estivoautumnal tertian. Should Koch's observations be confirmed in other tropical countries, we shall have to consider this type of fever as the most diffuse and the most important of all.

Some differences existing between the clinical phenomena of our estivoautumnal fevers and those of tropical fevers appear to us to relate to the gravity and perhaps the frequency of some of the complications rather than to the disease itself. The albuminuria so frequently found by Marchoux is very rare with us; moreover, it was also rarely found by F. Plehn. Sunstroke and perhaps pyretogenous infections are also frequent complications in the tropics, but rare with us.

Even the pernicious forms of the disease present the same symptomatology as those observed in our own climates; the comatose type appearing to be everywhere the most frequent. The chief difference, however, between malaria found in temperate climates and that of the tropics is the great frequency of hæmoglobinuria in some parts of the tropics, as the West coast of Africa; while with us, as is well known, although the condition does sometimes occur, it is so seldom that we scarcely need to take it into account.

Mixed Infections.

So far we have spoken of the various kinds of malarial infections to which are related definite species of parasites. In these cases the infection is pure in its type. There are cases, however, of mixed infection in which several species of parasites coexist. The combination may be of the ordinary tertian and the quartan, or of one or both of these with the estivoautumnal. Golgi was the first to give examples of mixed infection, mentioning among others a variety in which there were three generations of quartan and two of tertian parasites.

If each kind of parasite completes its whole life cycle, then according to the number of generations there will result certain irregularly intermittent and subcontinuous types of fever quite unlike irregular fevers of pure type. In practice, however, these singularly complex curves are rarely met with as a result of the coexistent parasitic species, but one kind usually obtains the ascendency and stamps its character upon the fever.

In estivoautumnal fevers it is not unusual to find a few ordinary tertian or quartan parasites mixed in with the ones special to the disease, but they exercise no influence upon the type of fever, and soon disappear from the blood as if overcome by the superior strength of the others. Di Mattei's experiments bear out this statement; he found that inoculation with estivoautumnal parasites in the blood of a patient affected with quartan fever caused the disappearance of the quartan parasites and the development of the ones inoculated; just as the inoculation of quartan parasites in blood infected by estivoautumnal parasites caused a disappearance of the latter and a multiplication of the quartans. This phenomenon of the removal of one species of parasite when another begins to develop occurs also when several species coexist in the blood without inoculation. Somewhat rarely we have mixed infections in which the various species are each pathogenically active. Thus in the cyclic course of the tertian parasites we may see that of the quartan, and in the cyclic course of the estivoautumnal that of the tertian or the quartan. In a case which came under our observation, when the attack due to the estivoautumnal parasites came to an end, by subintrance there came one occasioned by quartan parasites whose sporulating forms were found together with the pigmented forms of the estivoautumnal.

When quartan or tertian parasites are found in chronic estivoautumnal infections at the period when crescents alone are present, it is evident that the febrile type to which these correspond will remain undisturbed; but it will become irregular if the febrifacient stage of the estivoautumnal parasites appears.

A microscopical examination of the blood, by demonstrating the various species of parasites and the number of generations present, will explain irregularly intermittent or subcontinuous mixed infections.

Chronic Malaria.

Malarial infection may be acute or chronic in its course—acute, when after a variable number of attacks a cure occurs either spontaneously or following the administration of quinine; chronic, when

the infection of the organism continues for months and even for years. We must distinguish, when possible, between cases in which the chronic condition followed upon the first infection and those in which there has been a succession of infections.

Chronic malaria is manifested by febrile attacks which are repeated at greater or lesser intervals, by enlargement of the spleen and liver, and by a secondary anæmic condition and its results. In malarial districts chronic infection is readily recognized by a special earthy coloration, an enlarged abdomen, and torpidity and depression of spirits. Sometimes, however, in genuine chronic infections, especially if the fever is mild and if the attacks are repeated at long intervals only, the patients may be in a good condition, with the exception of slight and transitory anæmia after attacks, and may attend to their occupations with their usual activity. In the hospitals we frequently have occasion to see young men of robust constitution with a fine muscular development, hands hardened by toil, and bronzed complexions, who complain of having had occasional attacks of intermittent fever for years; and at the autopsies of some of them who have died of acute diseases, especially pneumonia, we find a melanotic enlargement of the spleen and perilobular melanosis of the liver.

Relapses.

Although all physicians practising in malarial countries agree that chronic infection may be manifested only by anæmia and splenic enlargement, or even by the latter symptom alone, without fever, yet in the majority of cases the fundamental symptom consists in a repetition of the febrile attacks—in other words, of relapses.

The intervals separating the several relapses are of variable duration. When the fever of the first invasion has gone, either spontaneously or after the administration of quinine, it returns usually after five, six, nine, twelve, fifteen, twenty, or even more days of apyrexia; or the relapses may occur only after months or a year have passed. This we know from the fact that persons who have suffered from malaria, after living for months in a place which is absolutely non-malarial, may have a return of fever of the same type as the first.

A return of the fever is favored by the same conditions, or rather occurrences, which favor the first development in those living in a malarial region, such as chilling of the body,* especially during per-

* Even Horace knew that relapses of intermittent fever are promoted by a chilling of the body, as may be seen from the following verses (Satires III., Book 2,

spiration, the abrupt passage from a warm to a cold climate, dietetic errors, overwork, bad or insufficient food, mental suffering, and intercurrent diseases (pneumonia, bronchitis, influenza, traumatism, puerperal fever, etc.). The first two explain the relapses which occur after a sudden chilling of the atmosphere or a cold bath, and the prompt return of the fever when patients go from malarial plains to mountain heights, without taking the necessary precautions. Relapses, as all physicians know, may occur in every form of malarial infection—quartan, ordinary tertian, and estivoautumnal. Recent researches have confirmed this fact by demonstrating in the relapses the special varieties of parasite.

Relapses with quartan parasites were observed by Golgi, and then by Antolisei, Vincenzi, and many others. Occasions to observe them are not lacking in hospitals in any malarial region. Thayer refers to a case in which three or four febrile attacks with quartan parasites occurred at intervals of exactly eight days. Relapses with tertian parasites are also frequently seen. In a case of Bignami's, there were groups of two, three, and even four febrile attacks which ceased spontaneously and were separated by intervals of fourteen, fifteen, and sixteen days of apyrexia. In another case seen by ourselves, the tertian infection lasted about two years, the groups of attacks occurring at intervals of a month. Vincenzi also observed relapses with tertian parasites which were repeated at intervals of about a month.

Relapses with estivoautumnal parasites are usually seen in regions of grave malarial infection. When the disease is contracted in the summer or in the autumn, after grave or pernicious attacks it is rarely brought to a sudden end, but is apt to relapse through the winter up to and even beyond the following spring. And it is to be noted that these relapses occur not only in men who are obliged to remain and to work in the malarial regions where they first became affected, but also in those who leave the affected district. We have known cases of malaria contracted in the Pontine marshes which relapsed when the patients were high up in the Engadine.

Estivoautumnal relapses may be regular almost to periodicity,

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lines 288 to 295) expressing the prayer to Jove of a mother whose son is suffering from quartan fever:

> Juppiter, ingentes qui das adimisque dolores,
> Mater ait pueri menses jam quinque cubantis,
> *Frigida* si puerum *quartana* reliquerit, illo
> Mane die, quo tu indicis jejunia, nudus
> In Tiberi stabit. Casus, medicusve levarit
> Ægrum ex præcipiti; mater delira necabit
> In *gelida fixum ripa, febremque reducit.*

but they are more often irregular, the attacks being of varied intensity and duration, usually occurring in groups of two, three, or five; the series of attacks being separated by intervals of apyrexia of six, eight, fifteen, or more days—intervals in which may occur isolated and usually short and light attacks. The type is quotidian or tertian or irregular; in summer and autumn we may see the relapses of a malignant tertian with a repetition of the same febrile curve. The quotidian type predominates in winter relapses, but the quotidian attacks sometimes alternate with the tertian. The parasites are the same as in the primary infection: small, without pigment or with very fine granules, and also crescent forms; the latter may be absent, but when they are present, as is usually the case, they are found alone only during apyrexia. These crescent forms, while they may be vacuolated and disintegrated, free or within leucocytes, may also be found as young endoglobular forms in the peripheral blood, but more especially in the blood of the spleen, whence it is certain that there is a continual formation of crescent bodies to replace such as disappear by disintegration in the blood or from the action of the phagocytes. At periods when crescents are abundant it is also usual to observe in the specimens the formation of flagellate bodies with the characteristics already described. At the near approach of the relapses the crescent forms diminish or disappear, while bodies of the febrifacient stage appear and multiply in the blood, to be later replaced by crescents during the period of apyrexia. In winter relapses the small non-pigmented parasites are either motionless or only slightly motile, and are discoid and annular; in the late relapses they may be extremely scarce.

The symptoms accompanying these relapses are the same as those described in connection with the various febrile types, although as a rule they are less severe. We sometimes have pernicious symptoms with a fatal issue, but these are relatively more frequent in the autumnal or first relapses, and very rare in the winter. It sometimes happens that in the relapse the predominant symptom of the primary infection will be repeated, as vomiting, diarrhœa, cardialgia, etc. In relapses of pernicious fever there is occasionally a repetition of the grave syndrome of the first attack. Thus we have seen a relapse of algid pernicious fever occurring about two weeks after the first attack was cured by means of quinine.

We have so far considered relapses of the single varieties of malarial infection. We may add that we have also observed relapses in which there was an alternation of the various malarial infections in cases in which a fresh infection from without could be absolutely excluded, as for instance when the patients lived in the city in the win-

ter or were for a long time in the hospital. Under these conditions was observed a relapse with ordinary tertian parasites following a quartan infection, succeeded again by the tertian, and then by a mixed infection of quartan and tertian, and finally by the tertian (Vincenzi). If the intervals separating these various relapses are not long, an examination of the blood will show that while one parasitic species, as the quartan, is gradually diminishing along with a diminution in the febrile attacks, the ordinary tertian parasite will appear in the blood and will increase until it produces its special febrile attack (Bignami and Bastianelli). Tertian and quartan relapses have been seen to follow estivoautumnal ones and *vice versa*, as also alternations of mixed estivoautumnal and tertian, and pure tertian or quartan. In these cases there is no warrant for believing in the transformation of one species of parasite into another, but we must believe that the infection was a mixed one from the beginning, and that the various species alternate in the relapses.

Whatever the origin of the relapses, provided there be no new infection, they usually become less and less severe; that is to say, the intervals are longer, the attacks are shorter, with a lower temperature and lighter symptoms; sometimes the patient is scarcely conscious of them, and finally they stop entirely. This gradual descent, as it were, in the attacks is seen especially in the winter relapses which, even when not treated, die out in the spring. This was well known to the older physicians, who advised patients suffering from winter relapses of fevers contracted in the autumn to trust less to medicines and more to nature and the approaching spring season.

After having seen that relapses may occur in all kinds of malarial infection and may even be of long duration, we naturally are inclined to ask, What is their genesis? Up to the present time the secret has escaped all direct observations. But by a process of induction from known facts we are able to form an idea of what takes place. After either spontaneous or medicinal recovery the parasites are not seen in the blood, or else only the non-febrifacient forms are present; but the fever-producing parasites return with fresh attacks. Now since, in the majority of cases, the duration of the interval of apyrexia corresponds to that of spontaneous or even of experimental incubation, we are led to believe that the time between the first invasion and the relapse and that between the single relapses should be considered as periods of incubation—that is to say, as the time which such parasites as have been able, on account of their special resisting-power or for some other reason, to survive the action of quinine and the defensive powers of the organism find necessary to increase to the number

needed to produce an attack of fever. This interpretation explains the varied duration of the periods of apyrexia; the length of this period of apyrexia or of incubation will be in proportion to the number of parasites which have managed to survive, as may be seen in experimental cases of malaria according to the amount of matter inoculated. Bignami and Bastianelli, who uphold this interpretation of relapses, call attention to the confirmatory fact that the quartan and the single, double, and triple tertian often relapse with the same variety in the type, or the same number of parasitic generations. This could not occur unless a certain number of each generation of parasites survived and preserved its rhythmical individuality during the period of apyrexia.

But to account for relapses which occur after intervals of apyrexia much longer than that of incubation, not only must we assume that there is a scanty number of parasites, but also remember the fact (until now ignored in malaria, but really existent as proved by spontaneous cures) that by means of the defensive powers of the organism the multiplication of the surviving parasites is limited to a very small proportion, and that only after a struggle for existence do they succeed in attaining sufficient numbers to cause a relapse of the fever.

The alternation of relapses with different distinct species of parasites shows that several or all may lodge in the organism at the same time without always coexisting in the blood or producing fever. It is worthy of note that while one species of parasite disappears from the blood, another appears and infects it. Why does infection die out? Is it because the powers of the organism prevent the parasites from multiplying further in the blood? But if these powers suppress one species of parasite in the blood, it must be acknowledged that they leave the field free to another species, because the latter multiply until they are sufficient in number to produce the fever. Is it due to a prepotency residing in the invading species of parasite? Even were that the case it is also true that this victorious species is in its turn overcome when the first variety returns and invades the blood. Is it due to the impossibility of the coexistence of several kinds of parasites in the blood? But we know that there are primary infections and relapses with several species of parasites in the blood. Is it a spontaneous weakening which occurs in the parasite after a certain number of generations? But why should this occur? This is a problem which deserves careful study, as it relates not only to everyday practice and to the question of immunity, but is also of deep scientific interest.

We should make a distinction between relapses and recurrences which begin two or three days after the primary infection, especially

if quinine has not been given in sufficient amount; in this case the larger number of residual parasites quickly attain sufficient proportions to produce the fever. No essential difference exists between the two—it is merely a question of the number of parasites. In recurrences there is a speedy return of the fever, just as after inoculation with an abundant amount of blood rich in parasites a first attack is rapidly induced. We maintain the distinction in malaria as we do in the case of other infections chiefly because of its interest practically; for it is well that the physician should know of the possible quick return of the fever, which in estivoautumnal infections may be pernicious.

We have already stated that relapses may occur after months or even more than a year. This was known to the older physicians and has been again confirmed recently. Relapses of quartan have been noticed after nine and ten months of apyrexia in patients who had never left the hospital in that time; we once observed a quartan relapse after a year's interval in a patient suffering from paraplegia of traumatic origin, in the last weeks of his life, when he had decubitus, cystitis, and pyelonephritis, which led to the fatal issue. Thayer tells of a physician living in a mountain region who had a relapse of tertian fever eighteen months after the last previous attack.

The explanation of these relapses after so long an interval cannot be the same as that given for ordinary relapses. We must suppose a latent inactive infection, as in other diseases, for instance syphilis, in which at a given moment the infection lights up and becomes manifest. Where and how is the infective agent preserved during so long a period? Bignami's theory, accepted by Bastianelli, is the only explanation so far given of the phenomenon; it is as follows: The spores of malaria are frequently enclosed in leucocytes, and although not provided with a membrane, they are of the extraglobular forms, the ones which preserve their structure and their capacity for staining the longest of any, thus showing themselves to be possessed of greater resistant powers than the others. Now *a priori* nothing would seem to oppose the idea that some of these spores, born like the others without a membrane, might finally acquire one, and by so doing acquire resistant powers and lose the property of staining. From them might originate the parasitic generation which causes the long delayed relapse. But this, as we have said, is merely a theory, and it will be no easy task for investigators to show where and how the parasite is domiciled in all this long period of latent life.

Long-Interval Fevers.—Before leaving the subject of relapses,

which, as we have seen, can occur in every variety of malarial infection, it will be advisable to return briefly to the subject of the existence of febrile types the attacks of which are developed at intervals longer than those between the attacks of quartan, and which therefore are in relation with the life cycles of other species of parasite.

It is true that in works on medical pathology, as we have seen when discussing the classification of fevers, there are mentioned fevers which in comparison with the quotidian, tertian, and quartan the older writers called "febres longiora intervalla habentes" (Burserius), that is to say, a quintan, sextan, septan, octan, nonan, deciman, quattuordeciman, etc., occurring every five, six, seven, etc., days. But as we have seen, relapses with intervals of apyrexia longer than the quartan occur in every kind of infection, and up to the present time no special variety of parasite has been discovered whose life cycle lasts four, five, eight, ten, or fifteen days. Golgi indeed, after having described the parasites of the quartan and the tertian and called attention to the differences between them, described fevers recurring at intervals of five, six, eight, ten, twelve, and even fourteen and fifteen days, and held that they were related to the life cycle of the crescent forms; for this cycle is not completed at a constant period, but is one of variable duration, differing in different subjects and even in the same subject under circumstances not very well defined. From these crescent forms would come the small non-pigmented bodies, which would invade new red corpuscles, and after having acquired fine granules of pigment would disappear in twelve, twenty-four, or thirty-six hours; during the long periods of apyrexia there would be a variable number of bodies of the crescent phase, which would pass from round or oval shape to the semilunar, and when their development was complete they would produce new small bodies which would invade the red corpuscles, causing fresh febrile attacks. But how are these fevers with longer intervals to be distinguished from the relapses of the estivoautumnal fevers? The parasites are the same; their behavior is the same; they are non-pigmented or have granules of pigment during the febrile period, and are crescentic during the intervals of apyrexia. The febrile type is the same, the apyretic periods are of the same duration, and the sporulation of the crescents, which is the fundamental postulate, is as yet a mere supposition which has not been demonstrated; indeed recent investigations have shown beyond doubt that the bodies of the crescent stage, sterile in man, are destined to ulterior development in the bodies of certain diptera which suck human blood.

Therefore we conclude that the fevers, chiefly winter fevers, following infections contracted in the summer and autumn, which occur

at intervals of variable duration with a frequently quotidian type and in groups of attacks (during which we find the small parasite without pigment or with very fine granules, with or without crescent forms, which latter, when they are present, occur alone only during apyrexia), are relapses of the fevers produced by the estivo-autumnal parasites, and collated to the relapses of quartan and tertian fevers.

Chronic Enlargement of the Spleen and Liver.

In addition to the febrile attacks, enlargement of the spleen and liver is an almost constant symptom of chronic malarial infection. The size of the splenic tumor depends chiefly upon the duration of the infection, but seems also to be influenced by other factors, such as the individual malarial district, the age, and possibly certain more personal and individual reasons which are as yet unknown. In infants and in children it is formed more rapidly than in adults, and speedily attains large proportions, while in the aged it neither develops rapidly nor becomes of large size. In old malarial patients it may become enormous, so that a large tumor may be found at the physical examination occupying the left hypochondrium and invading a good part of the corresponding half of the abdomen; its anterior margin is irregular, and it is seen to be displaced during the respiratory movements, this serving to distinguish it from more deeply situated neoplasms of the abdominal cavity. The liver is also regularly enlarged, and the lower border is felt to be thickened and rounded. The patients frequently complain of pain in the hypochondria, especially the left; this is nearly always the result of perisplenic and perihepatic inflammations, which occasion the frequently tenacious adhesions between these viscera and the parietal and diaphragmatic peritoneum.

Anæmia.

An anæmic condition accompanies chronic malarial infection, as evidenced by pallor of the skin and mucous membranes, the macroscopic appearance of the blood, and the diminution in the number of red blood corpuscles and in the amount of hæmoglobin. Weakness, apathy, fatigue, vertigo, palpitations, and torpidity of the digestive functions are the results.

The anæmia is most severe in the estivoautumnal affections; with every relapse there is a fresh reduction of the red corpuscles, although in a comparatively smaller degree than in the first infection. If the relapses are frequent, if the patient is not living under hygienic conditions, if his food is insufficient and the digestive powers are impaired,

then there will be established that anæmic state which is so frequently seen in malarial regions, often accompanied in children by anasarca, and which is an open doorway to other infections.

If in anæmic patients relapses with grave attacks occur, with many parasites, then by reason of the great destruction of red blood corpuscles the anæmia may become so threatening as truly to deserve the name of *pernicious* (Bastianelli and Bignami). The patients are not in coma, but they are profoundly depressed, apathetic, with a thready pulse and waxy skin, like persons rendered anæmic from grave hemorrhages. In these cases, to save the patient's life, the specific remedy will not be enough, but we must endeavor to make up for the loss of red corpuscles by transfusion of blood according to Ziemssen's method, as was done once by Bastianelli and Bignami with good results. But even if the attack is overcome, the parasites are driven away, and the destruction of corpuscles is arrested for a time, *an acute post-malarial pernicious anæmia* may supervene and carry off the patient.

In these grave secondary anæmias an examination of the blood shows in some cases nucleated red corpuscles, usually normoblasts, larger red corpuscles which are stained greenish-blue by methylene blue, an increase in the blood plaques, and a diminution in the number of white corpuscles. The presence of nucleated red corpuscles in the circulation is a sure indication that the functions of the hæmatopoietic organs have taken on increased energy in order to make up for the red corpuscles destroyed by the infection, the reparatory process going on with such rapidity that red corpuscles which have not lost their nuclei penetrate into the circulating blood.

In the spring infections, and even in the estivoautumnal ones, when the intervals of apyrexia are long and the febrile attacks not numerous, the anæmic condition is slight and transitory, because the blood is reconstituted during these periods of apyrexia, especially if the patient be living under good hygienic conditions. Indeed, we see well-nourished patients who are under tonic ferruginous treatment gradually improve until they have a normal amount of hæmoglobin, although febrile attacks continue to recur at long intervals.

In chronic malarial infection with persistent anæmia there is often slight albuminuria with polyuria and wandering œdema. The albuminuria may be continuous, or it may be or may become intermittent, in which case it occurs after the febrile attacks, with polyuria; during the fever the urine is scanty, highly colored, and throws down a copious sediment.

The hydræmic condition accounts for the facility with which hemorrhages occur in some malarial patients, epistaxis being the most

frequent form. Some chronic malarial sufferers have hemorrhages from the nose and into the skin with every febrile attack. Bignami and Bastianelli have described a case of chronic infection in which for four months the patient, who was anæmic, had not only irregular attacks of fever but periods of several days during which he had hemorrhages; these were seen on the skin of the trunk and limbs, came from the mucosa of the nose, were abundant on the tongue, the soft palate, and the gums; blood was also found in the dejecta, and there was hemorrhage from the ear. The above-mentioned authorities incline to think that in this case there was some complicating hemorrhagic dyscrasia or infection, because the hemorrhages continued to be abundant after quinine had stopped the febrile attacks, and because repeated examinations of the blood showed a scarcity of blood plaques, which is a constant sign in purpura hæmorrhagica (Denys and Hlava), while in malaria there is an abundance of blood plaques. But it is well to recall that in some patients hemorrhages continue even after the malaria has been overcome; epistaxis, as we have already said, is the most frequent form.

Neuralgia.

The intermittent and even periodical neuralgia of which the patients complain has been held by practitioners to be an effect of the active infection, that is to say, *true concealed or larval intermittent fever*. According to some, this may be the only manifestation of the disease in persons living in malarial climates. This concealed malaria has been much abused by clinicians in the past, some of them holding that malaria may occur under the disguise not only of neuralgia, but also of other symptoms, such as paralysis, contractures, hemorrhages, diarrhœa, renal colic, hepatic colic, etc., but since the discovery of the parasite, no one has described intermittent neuralgia or colic with parasites in the blood. Of the true concealed nonfebrile infection we have already spoken when discussing pernicious fevers, of which the proof is afforded by the presence of many parasites in the blood. We cannot accept the fact that these intermittent neuralgias are cured by quinine as a proof of their malarial nature, because we cannot overlook the other fact that we obtain the same results with the same remedies in cases of neuralgia from which malaria can be positively excluded. However, although it is very doubtful whether the intermittent neuralgias are due to localization of the parasites, yet it is true that persons suffering from malaria are apt to have, in addition to the febrile attacks, frequent neuralgias not only in the field of the trigeminal and especially the supraorbital branch, but also of the sciatic, the crural, the intercostal nerves, etc.

SEQUELÆ.

In malaria as in other affections we frequently have occasion to observe that when the infection has ceased it is succeeded by other diseases. Nor should this cause any surprise when we consider the grave alterations of the blood and of the hæmatopoietic and depuratory organs, the lesions of the nervous centres, of the digestive apparatus, and even of other organs which occur in grave infections, and lastly the probable intoxication caused by the parasites, by the products of their destruction and of that of the erythrocytes and the phagocytes, and by the disturbance in metabolism.

Of the morbid sequelæ of which we shall speak, the connection of some with malaria is evident, but of others it is necessary to explain the genesis by the admission of some intermediate factor, whence the difficulty in these cases of distinguishing between sequelæ and complications. The latter are at the most favored in their development and modified in their course by the malarial infection, while the sequelæ are so closely connected with the disease that in any given case we must conclude that without this disease they would not have developed at all.

Although strictly speaking the term sequelæ should be reserved for those affections which develop only after the acute infection has stopped, or after it has lasted some time, or which are related to organic changes due to malaria, yet we include among them those symptoms which originate during the infection and continue after the disappearance of the parasites.

We will now describe the chief morbid sequelæ.

Nervous Diseases.

We have already spoken of the nervous phenomena which accompany pernicious malarial infections, but even in less severe malaria certain nervous symptoms may occur, such as inequality in the pupils, unilateral or bilateral inferior facial paralysis, deviation of the tongue, hyperæsthesia from compression of the nerve trunks, and even symptoms of polyneuritis. These symptoms sometimes vanish when the infection ceases, that is to say when the parasites disappear from the blood, but sometimes they remain for days and weeks, only gradually disappearing. Thus we often see patients convalescing from grave malarial infections who appear to have a good appetite, and to be no longer very anæmic, and yet who continue to have paresis of the lower part of one side of the face, or slight dysarthria. or cerebellar ataxia,

or psychical disturbances, or aphasia. Following grave malarial attacks when the patients remain very anæmic we sometimes observe for days a condition of cerebral stupor or of tranquil delirium, or other psychical disturbances such as melancholia, hallucinations, and amnesia. Kelsch and Kiener report disturbances of this nature which persisted for several days after attacks of epileptiform malaria.

Nervous symptoms of various kinds may begin after the parasites have all disappeared from the blood; these are truly *postinfective*, and according to the descriptions given in the well-known work of Kahler and Pick upon acute ataxia following acute infectious diseases, which apply to post-malarial nervous phenomena as well, some run a rapidly fatal course, others keep on for days or weeks or longer, and are then cured, while still others either persist and remain stationary or become gradually worse, or are only partially cured.

To the first category belong the cases of *delirium* which occur after soporific or comatose pernicious fever and end fatally. We recall several instances, among others that of a youth in whom an attack of comatose pernicious fever ceased and was followed by a day of prostration without fever and with the presence of crescent forms only in the blood, during which there arose grave delirium with hallucinations which so excited the patient that he had to be held in bed by force; the delirium lasted for three days, and ended in death which was preceded by a short period of hyperpyrexia. At the autopsy the brain was found to be very pale; in the cerebral capillaries, many of the endothelial cells of which contained pigment, there were no parasites, but many crescent forms were found in the bone marrow.

Such lethal postinfective nervous symptoms are most rare; more frequently we have those which gradually improve until there is full recovery. *Hemiparesis, monoparesis, aphasic disturbances,* symptoms which recall those of multiple sclerosis, cerebellar uncertainty of gait, psychical disturbances, etc., may all occur in patients convalescent from grave malaria which has caused much destruction of the red blood corpuscles. Ferraresi observed two cases of postinfective psychoses in malaria. One of them was that of a woman thirty-five years old who had contracted the fever in Ostia. After an attack of protracted soporose pernicious fever she remained very anæmic and profoundly melancholic, thinking herself abandoned by everybody. A period of exaltation followed, during which she refused to take food, and thought that an attempt was being made to abduct the daughter who was with her, and endeavored to escape from the hospital in order to remove her from persecution. With an improvement of the blood state the psychical disturbances disappeared.

Even *multiple neuritis* as a sequela of malarial infection has been

described by various neurologists, as Gowers, Pitres, Macnamara, and Raymond. Gowers states that he has noticed that persons living in malarial regions have suffered from weakness of the lower limbs, more particularly in the muscles of the feet, and especially in the anterior tibial group of muscles. In some cases there was true paralysis of a degenerative nature in these muscles, followed by contracture of the muscles of the calf, a result of the falling of the foot; but when the patients finally succeeded in standing on their feet, the contractures disappeared. Gowers believes that this is a neuritis due to malarial infection or secondary intoxication.

Glogner describes six cases of polyneuritis seen by him in Samarang (Java) which occurred during or after malarial infection, and distinguishes the affection from beriberi, which is endemic in that region. In the four cases which developed during the infection, that is to say when there were positive results from the blood examination, there were noted diffuse and often excruciating pains in the lower extremities, formication, pain upon pressure of the nerves and muscles, motor weakness in the legs, with the deep reflexes sometimes present and sometimes abolished, diminished response to electrical stimulation in the nerves and muscles of the lower limbs, partial or total absence of sensibility, and sometimes œdema of the legs. Some of the patients were anæmic, in all positive results were obtained from an examination of the blood, and all recovered after two or three months. The same symptoms and the same favorable termination were noticed in two cases observed by Glogner, the symptoms of multiple neuritis occurring a short while after the malarial infection had ceased. From Glogner's report it would appear that malarial infection is more apt to cause polyneuritis in those countries where beriberi is endemic; this is an infective disease whose fundamental lesion is a polyneuritis which affects not only the nerves of the extremities, but also those of the viscera, especially the vagus, whence we have dilatation of the heart, dropsy, and death from syncope. Scheube, however, thinks that the cases described by Glogner were complications of beriberi with malarial infection. But his opinion has not much weight when we consider that even in countries where berberi has never been known, as in the Roman malarial zone, cases have been observed of polyneuritis developing during or after the malarial infection. In the hospitals of Rome Chiarini and Bardellini have recently studied the polyneuritic syndrome. The former observed it in a young man of twenty-three years, who having contracted the fever in Ostia in June, 1895, had relapses up to November, accompanied by constantly increasing weakness of the lower limbs; in the last relapse, occurring in the early days of

November, there was an increase of the weakness, with pain which finally prevented walking. After a few days of rest the improvement was sufficiently marked to permit of his taking a walk, but the pain and motor weakness of the lower limbs returned, with added symptoms of weakness of the trunk muscles, slight nystagmus, slight dysarthria, and diminished electrical excitability. Subsequent improvement was followed by recovery, but the patient for a long time was weak and easily tired.

In Bardellini's case the patient was a young and robust driver who contracted malarial fever in the summer of 1896. On the 12th of August of the same year, after a severe attack of fever which subsided during the night, he began to have formication in the limbs and trunk, pains in the limbs, and motor weakness which increased from day to day until he was finally unable to move. In an examination made on the 21st of August the following symptoms were noticed: Paresis of the left side of the face, left pupil larger than the right, weakness of the muscles of mastication, deglutition interfered with, general motor weakness making it impossible for him to rise or even to sit; the only movements possible in the lower limbs were those of adduction and abduction of the foot; the patellar reflex was abolished, the cremasteric and abdominal reflexes were intact; there was sharp pain upon pressure of the nerve trunks; there were also pains diffused throughout the body, and paræsthesia of the limbs; sensibility was normal. This condition continued until the 25th, during which time the patient took only liquid nourishment. On the 27th there was a sudden attack of shortness of breath, orthopnœa with threatening suffocation, but stimulant injections and inhalations of oxygen averted the danger of asphyxia. A few days later an improvement began, which was frequently interrupted by worse conditions and by malarial relapses, but which finally became progressive.

A case has been described of pernicious infection with the symptoms of *electric chorea*. Recently Chiarini has observed that this affection may begin during a febrile attack and continue for months after the infection has ceased. This is exemplified in the following case which he studied, and of which we give a *résumé:*

E. M——, a countryman, 21 years old, contracted the fever in August, 1895, and had relapses up to the 5th of December; on that day, during the febrile attack, choreic symptoms began and continued with increasing severity for several days, unaccompanied by fever and in spite of the administration of quinine, and became so troublesome as to prevent his working and obliged him to repair to the hospital. His condition was the following: malnutrition, pallor, splenic tumor, in the blood a few crescent bodies, nystagmus of an irregular and abrupt nature, with intercurrent strabismus and rotation of

the eyeballs, increased under fixation; rapid, brusk, and disordered movements of the orbicular muscles and of those of the face and the neck, especially on the left side; rapid and rhythmical clonic movements of the tongue causing dysarthria; very active cutaneous reflexes (merely touching the patient excited active clonic spasms) and exaggerated deep reflexes; marked depression; somnolence. On the following days, the apyrexia continuing and the crescent bodies having disappeared, there was atrophy of the muscles of the neck, trunk, and limbs, the patient being unable to sit up in bed; the appetite was voracious, and there were long periods of sleep. From the 21st of December there was improvement; the patient could sit up in bed, but if he attempted to get up he was seized with violent clonic contractions of the muscles of the trunk and limbs. By the middle of January there was great improvement; the patient walked with long and bounding steps; the dysarthria had disappeared; nutrition had improved; the nystagmus occurred only under fixation; the appetite continued to be voracious—a true boulimia. At the end of February the patient was pronounced cured.

From the clinical data which up to the present time have been at our disposal, we are unable to judge with certainty whether the nervous diseases developed during or after the infection can become chronic or remain only partially cured. There are some written accounts of cases of multiple neuritis which, beginning during or after the infection, remained stationary for several months. In practice it is not unusual to observe nervous disturbances such as neuralgias, motor weakness, and easily induced mental fatigue in patients who have only recently recovered from malaria; but whether a cure followed or not we are unable to say. We remember having observed the case of an old woman with right hemiparesis which had appeared after the second attack of a comatose pernicious malarial fever that had been overcome with difficulty three years before.

In regard to the genesis of these nervous symptoms, we must bear in mind the lesions which pathological anatomy has demonstrated in pernicious malarial infections, that is to say the punctiform hemorrhages, and the lesions which the microscope has shown us in the capillaries, the protoplasm, the nuclei, and the prolongations of the nerve cells. Sometimes the punctiform hemorrhages are found over the whole of the white substance of the brain; again they are limited to certain areas of the centrum ovale or of the crura cerebri, the ganglia, the internal capsule, or the medulla oblongata; or else they are limited to the cerebellum and there to the gray substance. But even when these lesions are not visible, it is probable that by means of the intravascular and vascular changes produced by the febrile attacks, disturbances of nutrition are caused in the nerve cells which are not easily recognizable by the means at our command. In these demon-

strated or probable lesions we may find the cause of the nervous symptoms, those which persist after the infection as well as those which develop subsequent to its cessation. In the case of multiple neuritis, however, the theory of intoxication has a better foundation.

Finally we must add that in the aged with arteriosclerosis and miliary aneurysms of the brain, cerebral hemorrhages may occur during an attack of pernicious fever. This was probably the origin of the hemiparesis in the case of the old woman referred to above, which came on during the attack and was still persistent three years later. The same origin was suspected by Thayer in a case of right hemiplegia and aphasia occurring during a paroxysm of tertian fever in a man, fifty-three years old, suffering from arteriosclerosis, and which never completely resolved.

Diseases of the Eyes.*

Ocular complications are not common in malaria, yet they occur with sufficient frequency to merit attention in a treatise on this affection. It is especially within the tropics that such complications of malaria are observed, and among the different forms of malarial fever the estivoautumnal is the one in which eye troubles are most frequently observed. The parts of the eye most prone to malarial lesions are the retina and the optic nerve. Among the ocular affections of malarial origin we must distinguish those due to acute malaria from those encountered in the chronic disease.

Amaurosis.—A relatively frequent trouble in acute pernicious fevers is temporary or intermittent amaurosis or amblyopia. Even before the discovery of the ophthalmoscope certain observations of this sort had been made, and on them was based a form called amaurotic pernicious fever. But we cannot accept as genuine all the cases which have been published under the name of malarial amaurosis or amblyopia, for in many of them, especially the febrile ones, the malarial origin, affirmed solely by the fact that a cure follows the use of quinine, is more than doubtful. There are some cases, however, in which a temporary amaurosis or amblyopia is undoubtedly due to a malarial infection. Such cases are encountered more particularly during the course of grave estivoautumnal and pernicious fevers, and the eye trouble is frequently accompanied by other disturbances on the part of the nervous system. Pennoff, who made a study of the ocular troubles due to malaria in a regiment of soldiers stationed at Tiflis

* The section on diseases of the eyes of malarial origin has been kindly prepared by Dr. Giuseppe Chiarini, formerly assistant in the clinic for diseases of the eye at the University of Rome.

in the Caucasus, says that he himself, while suffering from intermittent fever, had for a period of twenty-four hours complete amaurosis of the left eye accompanied by aphasia and left-sided hemiplegia and hemianæsthesia. He says also that another physician, Dr. Reich, was taken one day during a febrile attack with bilateral right hemianopsia, which disappeared the following morning.

The amaurosis is most commonly bilateral and lasts from fifteen or thirty minutes up to ten hours or more; it generally shows itself during the initial chill, continues throughout the entire pyretic paroxysm, and passes away with the coming on of profuse sweating. There may be but a single attack, or the blindness may return in successive attacks and acquire an intermittent character. The type of the attack is for the most part tertian, and but rarely quotidian. When the attacks are quotidian and come on during the evening hours, the affection acquires an extraordinary resemblance to hemeralopia, and some writers have accordingly described a form of hemeralopia due to intermittent fever. Stöber relates an interesting case in which there first appeared a hemeralopia, and then after several days an attack occurred in full daylight and caused a complete bilateral amaurosis, which was cured after a few days by quinine.

During the amaurotic attack the pupils are dilated and respond sluggishly to light. Ophthalmoscopic examination usually reveals no apparent alterations in the fundus.

Koslorowsky found hyperæmia and œdema of the optic disc in a case of amaurosis coming on during an attack of intermittent fever and lasting several hours.

Malarial amaurosis has not always a temporary character, but is often persistent and may even terminate in permanent blindness through atrophy of the optic nerve.

Jacobi first described in 1868 a non-intermittent malarial amaurosis due to an optic neuritis which had the appearance of a choked disc. The visual acuteness of both eyes was much reduced, but the visual field preserved its normal limits. The continued use of quinine caused a disappearance of the subjective and objective troubles in four weeks. A similar observation has been published by Macnamara, who asserts that cases of this kind are encountered rather frequently among the natives of Calcutta.

In January, 1896, I had the opportunity at the Santo Spirito Hospital of observing a case of malarial amaurosis associated with optic neuritis, which came to autopsy. The patient was a man, 34 years old, an epileptic and a hard drinker, who, after having suffered several attacks of malarial fever, was suddenly taken with bilateral amaurosis, while the fever had disappeared. An ophthalmoscopic

examination, which was made early, showed an incipient optic neuritis. The pupils, which were somewhat dilated, remained immovable under the influence of light and during convergence. For two days the patient complained of nothing except the blindness; then he began to be feverish again and had motor and sensory paralytic symptoms appearing first in the lower extremities, then in the lower left side of the face, the upper extremities, and the trunk. At the same time appeared rectal and vesical paralysis, dysarthria, conjugate deviation of the head and of the eyes towards the right, and sopor becoming gradually deeper and terminating finally in complete coma. During the man's last hours bedsores appeared, and the temperature rose to 41° C. (105.8° F.). An ophthalmoscopic examination repeated the day before death occurred showed that the optic neuritis had progressed. The papillæ were slightly projecting with indistinct outlines, peripapillary retinal œdema was very apparent, the arteries were quite thinned, and the veins were turgid and tortuous. At the autopsy the evidences of an existing malarial affection were found. Examination of fresh specimens of the gray substance of the brain showed in the cerebral capillaries a certain number of red blood corpuscles containing malarial parasites with central pigment. Examination of the pulp of the spleen showed the presence of black pigment and crescent forms of the malarial parasite. On histological examination no changes were found in the nerve centres beyond the presence within the capillaries of red corpuscles containing malarial parasites all in the stage of central pigmentation. Histological examination of the eyes gave the following results: In the sheath, especially the pia and arachnoid, and in the connective-tissue framework of the optic nerve there was a small-celled infiltration which was progressively more marked the nearer the nerve approached its point of entrance into the globe of the eye. Even the optic disc, prominent and with a radiating striation more accentuated than normal, showed a small-celled infiltration which passed over for a short distance into the retina, especially in the layer containing fibres of the optic nerve. The capillaries and the veins of the retina were distended and filled with blood. The choroid was markedly congested, the large venous channels being enormously distended and gorged with blood; in the choriocapillaris was noted an accumulation of leucocytes (leucocytic stasis), in the midst of which were seen a few large phagocytes containing granules of melanotic pigment. No trace of malarial parasites was to be found in the vessels either of the retina or of the choroid.

I had occasion to see two other cases of malarial amaurosis with optic neuritis during the same year in the hospital of San Giovanni. One of these was in the person of a child and terminated in permanent blindness in both eyes in consequence of atrophy of the optic nerves. The other case was that of a woman who, after a couple of weeks of almost complete blindness, had a partial atrophy of the optic nerves, and was left with a moderate acuteness of central vision with a marked concentric limitation of the visual field. Both of these patients suffered from quite grave attacks of estivoautumnal fever.

Poncet, who was the first to study the pathological anatomy of the ocular changes due to malaria, has described a retinochoroiditis occurring with both acute and chronic malaria. Ophthalmoscopic examination revealed a venous congestion of the fundus accompanied by peripapillary retinal œdema and by a slight prominence of the optic disc, which had taken on a characteristic pinkish grayish-black tint in its central portion. Microscopical examination of the eyes of an individual dead during an attack of pernicious fever showed, in addition to the characteristic appearances of an acute optic neuritis with papillary œdema, an injection of the capillaries of the retina and optic disc due to a stasis of white corpuscles (?) containing black pigment in their centre and which were so crowded one against the other as to have taken on a polygonal shape. In the choroid the blood-vessels were found enormously distended and filled with leucocytes, pigmented for the most part.

Guarnieri, in a recent study of retinal changes complicating malarial infection, has corrected certain errors made by Poncet, who wrote in 1878 before the malarial parasite had been discovered. Guarnieri has shown that the retinal capillaries contain red corpuscles exclusively, and that these contain amœbæ which are most frequently pigmented. He asserts that there can be no doubt whatever as to the nature of these elements and that they cannot possibly be taken for pigmented leucocytes, as they were by Poncet. In the retinal vessels Guarnieri found few and slight changes. In some capillaries only he found the endothelial cells swollen with prominent nucleus and with very fine pigment granulations in the protoplasm. Degenerative or necrobiotic alterations were found very rarely. In the choroid Guarnieri found almost all the vessels full of large leucocytes with vesicular nucleus, rich in pigment, in granulations of hæmoglobin, and in red corpuscles containing plasmodia. The distribution of amœba-containing blood cells in the retinal vessels was made according to definite laws. The capillaries contained red cells with plasmodia in an advanced stage of their vital cycle; in the other larger vessels the red globules with adult parasites always occupied the peripheral portion of the blood column, just as Marchiafava and Bignami have described as occurring in other viscera.

Poncet and Guarnieri hold that even malarial amaurosis sine materia, in which ophthalmoscopical examination reveals no changes, is dependent upon these mechanical alterations in the blood circulation of the retina and choroid which they described. The explanation of the attacks of temporary blindness, says Guarnieri, is to be found in this globular and phagocytic stasis in the smaller vessels of these parts.

In addition to optic neuritis and retinochoroiditis, acute malarial infection is capable, according to certain authors, of producing other changes in the retina which may cause amaurosis. Thus Despagnet has described a case occurring in a young woman who had suffered in Africa from repeated attacks of pernicious fever, and on the fifth attack became totally blind. Upon the malarial attack being cured the sight did not return, and forty days later, in Paris, Despagnet discovered on ophthalmoscopic examination a generalized retinal arteritis. Under treatment with iodide of potassium and quinine the patient improved rapidly, and had finally relatively good sight, but with a greatly restricted visual field.

Retinal hemorrhages, with or without amaurosis or amblyopia, have quite frequently been observed in cases of acute malarial infection. Sometimes in consequence of their situation near the equator of the eye they escape detection by the ophthalmoscope, but they can always be found on histological examination of the eye post mortem.

The *diagnosis* of malarial amaurosis presents no difficulty, since malaria can hardly be mistaken for any other malady. In certain cases, however, there might arise a doubt whether the amaurosis was due to malarial infection or to quinine poisoning; and the need of a speedy diagnosis is of the utmost importance here, since in one case a cure would be quickly obtained by pushing the quinine, while in the other such a course would only aggravate the trouble. Quinine amaurosis could not be confounded with any other than the permanent form of malarial poisoning, since the former has always a fairly long course. In doubtful cases of this nature the ophthalmoscope will always decide the question. The ophthalmoscopic picture in cases of quinine amaurosis is most characteristic. There are signs of a grave ischæmia of the retina and optic nerve. The arteries and veins are extremely contracted, and all traces of them are often lost at a short distance from the papilla. The optic disc is from the first of a pearly whiteness, as in cases of atrophy of the optic nerve, and its contour is very distinct. There are no traces of retinal hemorrhage. Very different are the changes in the fundus as seen through the ophthalmoscope in cases of malarial amaurosis. There we find hyperæmia, papillary œdema, optic neuritis, and retinal hemorrhage or arteritis which give a picture wholly unlike that of quinine amaurosis. Sometimes, it is true, there is a narrowing of the retinal vessels, but this is of short duration, or when permanent is limited to the arteries alone and never occurs in the veins which are always more or less dilated.

In its subsequent course, also, malarial amaurosis differs greatly from the quinine affection. The former is almost always of brief du-

ration and often terminates in complete recovery, but may result in total blindness through atrophy of the optic nerve. Quinine amaurosis, on the other hand, is of much longer duration, but is of better prognosis, for even when complete amaurosis has lasted several months the patient, under appropriate treatment, will usually regain a fairly acute central vision. The visual field, however—and this is another characteristic of quinine amaurosis—is concentrically narrowed, sometimes to such a degree that patients whose field of vision may be sufficiently broad to permit of reading and writing cannot go out alone. Another characteristic of quinine amaurosis is that, even when the patient has regained a fairly good amount of vision, the ophthalmoscopic signs persist almost unchanged and the atrophy of the optic nerve contrasts strongly with the relatively good condition of the sight.

Other Ocular Changes.—Lesions in other parts of the eyes which are associated with an acute malarial affection have been described, but they are much less common than those of the retina and optic nerve, and we do not think their dependence upon malaria has been satisfactorily demonstrated.

Pennoff has described a case of *suppurative choroiditis* coming on during an attack of fever and terminating in atrophy of the globe of the eye. Cases of *iritis* have also been described by Pennoff. Selück saw, in a regiment in the Caucasus, in which seventy per cent. of all diseases were malarial, five cases of iritis with formation of posterior synechiæ which were cured in a few days after the administration of quinine. Ragot saw in Pointe à Pître in Guadeloupe two cases of soft *cataract* occurring in young mulattoes who had suffered from comatose pernicious fever. The opacity of the crystalline came on suddenly after the pernicious attack, and in each case a complete double cataract was formed in the course of a few months. Ragot holds that these cataracts were due to profound disturbances of the ocular circulation caused by the malarial infection. Van Milligen and Kipp have described a special form of ulcerative keratitis (keratitis dendritica) complicating malaria.

Eye Complications of Chronic Malaria.—The ocular changes encountered in chronic malaria are also located chiefly in the retina and optic nerve. Poncet has found in chronic malaria cases of retinochoroiditis with grayish-black injection of the median zone of the optic nerve, peripapillary œdema, and grave venous congestion, accompanied by very many retinal hemorrhages occupying in both eyes almost all the part accessible to ophthalmoscopic observation; these were minute and punctiform at the periphery, but large and irregular in outline in the neighborhood of the optic disc. In another case of

malarial cachexia, in which he was able to study the condition of the eyes under the microscope post mortem, Poncet found the retina thickly dotted with punctiform hemorrhages, but saw no melanæmia in the retinal capillaries, which, on the contrary, gave evidences of inflammation with proliferation of the endothelium. In the choroid also the melanæmia may be but little marked.

Sulzer, who had an opportunity of studying a large number of cases of malarial eye troubles in individuals who had contracted the fever in Java, Borneo, and Sumatra, frequently observed a chronic optic neuritis in those suffering from malarial cachexia. The most characteristic subjective symptom of this chronic optic neuritis, which is the same thing at bottom as Poncet's malarial retinochoroiditis, consists in marked oscillations in the degree of visual acuteness during the course of the disease. Diminutions in vision as low as one-tenth may improve so much within two or three weeks as to attain an acuteness of one-half or even two-thirds, then to fall again in the course of a few days to one-tenth. The affection is invariably bilateral, but does not ordinarily begin at the same time in the two eyes. The visual field remains intact or is at most very slightly narrowed concentrically. Color perception is always normal except in the cases which terminate in atrophy. In a certain number of cases Sulzer found hemeralopia. The ophthalmoscopic picture is that described by Poncet under the term malarial retinochoroiditis. The optic disc is prominent and has a grayish or blackish-red tint, the retina has lost its characteristic brilliancy in the parts surrounding the papilla, the contour of the papilla is indistinct, and the veins are dilated and tortuous. Sulzer lays special stress upon the color of the papilla as a diagnostic sign of importance. The same observer noted a termination in partial atrophy of the optic nerve with irregular narrowing of the visual field in eight per cent. of his cases of chronic optic neuritis. In about one-third of the cases he found peripheral hemorrhages of the retina, but absorption occurred promptly without leaving any pigment deposits behind.

Bull has described two cases of white atrophy of the optic nerves of malarial origin, without any signs of a preceding inflammation, with concentric limitation of the visual field, and dyschromatopsia.

Besides the hemorrhages accompanying malarial retinochoroiditis and chronic optic neuritis, others have been observed independent of these conditions in chronic malaria. Thus Pennoff saw in a case of malarial cachexia a number of small retinal hemorrhages accompanied by hemianopsia and dyschromatopsia, which occurred with every new febrile paroxysm. Von Kries has described two cases of intraocular hemorrhage occurring as a sequel of malarial fever; in one of these

cases there were multiple retinal hemorrhages in both eyes, and in the other case there was a severe hemorrhage in the vitreous of the left eye.

Serous Effusion into the Vitreous.—Seely in 1882, and more recently Sulzer, described a special change in the vitreous humor associated with chronic malarial infection. This consists in a whitish infiltration of the vitreous body, occurring at intervals and causing an almost complete loss of sight. On ophthalmoscopic examination nothing is seen beyond a white reflex characteristic of this affection. Seely attributes this change to a serous infiltration into the vitreous occasioned by chronic malarial poisoning. In the two cases described by him, after an oscillating course for a few months, the affection terminated in recovery, quinine having been given regularly. One of these cases was complicated with left supraorbital neuralgia, which disappeared together with the eye trouble; in the other case there remained permanently a few movable flocculi in the vitreous. In two of the cases observed by Sulzer the termination was more grave. These patients suffered from severe malarial cachexia with profound anæmia; the diffuse infiltration of the vitreous, which was already of long standing when the patients came under observation, was not completely absorbed in either of the cases, but gave rise to the formation of many movable flocculi joined together to form a sort of cobweb through the meshes of which the vitreous, still somewhat opaque, allowed the fundus to be seen only obscurely, yet with sufficient distinctness to permit the recognition of the atrophic white appearance of the optic disc. In a third case, which came under observation at the beginning of the trouble, Sulzer obtained a complete cure by the use of sulphate of quinine in large doses. Pennoff also described a diffuse opacity of the vitreous in cases of intermittent fever, but they were always associated with changes in the iris and choroid.

Albuminuric Retinitis.—Cases of amblyopia and amaurosis accompanied by œdema and albuminuria, in which occasionally the ophthalmoscopic picture of albuminuric retinitis has been noted, have been described as dependent upon malarial cachexia; but in these the retinal trouble is attributable rather to the renal lesion than to the direct action of the malarial poison. Poncet, who had the opportunity of making a microscopical examination of the eyes in one of these cases, found the lesions of ordinary albuminuric retinitis, complicated by special endarteritis ending in fatty degeneration, and also the presence of pigmented phagocytes in the capillaries of the choroid, this being the characteristic lesion of albuminuric retinitis due to malaria.

Diseases of the Ears.

Numerous affections of the ears have been described as due to the action of the malarial poison, but many reports of this nature should be accepted with reserve. Indeed, the intermittence of some auditory affection, its cure after the exhibition of quinine, or its occurrence in a patient coming from a malarious region who has an enlarged spleen, is not sufficient without other affirmative facts to establish its dependence upon malaria. There is no doubt that many affections which have been regarded by different authors as of malarial origin have been simply accidental complications. And again, in other cases, as we have seen when discussing malarial eye troubles, it is a question whether the disturbances should have been credited to the action of the malarial poison or to that of quinine. In the neighborhood of Rome aural diseases which can be regarded as dependent upon malaria are certainly very rarely encountered. In the aural clinic of the university, out of a total of about six hundred cases of ear disease annually not more than one per cent. can be credited to malaria; and this proportion would be still further reduced if we take away the cases of suppurative otitis media, which disease we believe to be an accidental complication and not the direct effect of malarial infection. In our long experience with malaria in the hospital of Santo Spirito we have never seen any auditory trouble beyond a more or less grave transitory deafness, except in very rare cases, and then only in patients who had taken quinine in large doses for a long period.

The following affections have been described by various otologists as of malarial origin:

Intermittent Otalgia.—Weber Liel (1871) and Voltolini (1878) have described cases of this kind which they regarded as instances of trigeminal neuralgia. De Rossi, of Rome, claims to have encountered this intermittent otalgia not infrequently in malarial subjects, the curative action of quinine being very evident. Politzer also asserts the existence of a malarial intermittent otalgia.

S. L. Frank, of Baltimore, has reported a unique case of intermittent tinnitus of malarial causation which was cured by means of arseniate of quinine.

Labyrinthine Vertigo.—Ferreri, upon whom we have drawn freely in the preparation of this section, reports two cases of this affection. The first was in the person of a patient who had suffered from malaria for two years, then recovered, and was again attacked with fever lasting for four months. During this latter period every febrile paroxysm was preceded by deafness and tinnitus, and then followed

extreme vertigo and retching. The vertigo was so pronounced that the patient felt as if he were falling down, and he would actually fall unless he sat down at once. With the appearance of the chill and fever the vertigo ceased, leaving the patient deaf and bewildered. Recovery took place at the end of about a month under treatment with quinine. The second case was that of a hunter who had acquired the disease at Sermoneta, a very malarious region, and had had two pernicious attacks. After many slight aural symptoms, in October, 1889, he was seized after a febrile paroxysm with deafness and ringing in the ears and with such severe vertigo that he fell to the earth, the face being drawn at the same time over to the right shoulder. This attack was repeated three times in the course of ten days, and the patient then consulted a physician, who found an enlarged spleen and estivoautumnal parasites in the blood; there was deafness, especially on the right side, together with bilateral tinnitus. A cure was effected in two months by means of quinine.

Certain lesions of the internal ear or auditory nerve, causing more or less deafness, which were apparently cured or improved under the use of quinine, have been attributed to the action of the malarial poison. Thus Wolff mentions a periodical deafness occurring in intermittent fever; and Gellé speaks of a special form of deafness which occurs in conjunction with febrile paroxysms and yields only to treatment with salts of quinine. This deafness may be the only manifestation of malaria or it may accompany the febrile manifestations; it is met with only in malarious districts, and all forms of treatment, except that by quinine, are said to be useless.

Thomas Barr says that the malarial fevers may often be followed by labyrinthine deafness, although he adds that the administration of quinine may probably contribute to the occurrence of this condition. Ferreri reports the case of a patient who, after two weeks of fever without having taken quinine, lost his hearing in the right ear and suffered also from marked tinnitus; otoscopic examination showed an apparently normal organ. In another case the same observer noted a loss of hearing on the right side following an attack of delirious pernicious fever in a lad sixteen years of age; objective examination revealed no disease of the middle ear.

Ferreri also describes two cases in which, during the course of a malarial infection with estivoautumnal parasites in the blood, deafness with vertigo and tinnitus occurred; the result of a careful examination in these cases was a tentative diagnosis of labyrinthine hemorrhage of malarial origin.

Otitis Media.—Finally we note that various otologists believe themselves justified in recognizing the existence of acute malarial

middle-ear disease, of which two forms have been described. One of these is characterized by a simple hyperæmia of the tympanum with a serous or mucoserous exudation; the other is a true purulent otitis media. To the first of these forms belong probably the cases mentioned by Weber Liel, who speaks of an intermittent malarial otitis, and those described by Hotz, who refers to patients prostrated by violent otalgia and cephalalgia and presenting all the symptoms of the most grave suppuration of the drum, while otoscopic examination revealed nothing beyond a very slight tympanic hyperæmia. In these cases the facts that the patients have come from malarious regions, the insufficiency of the lesions to explain the severe subjective symptoms, the inefficacy of the ordinary local treatment, and the curative action of quinine are sufficient, according to various authors, to prove the malarial origin. De Rossi, of Rome, claims to have observed not a few cases of suppurative malarial disease of the middle ear, and other otologists mention malaria among the causes of this affection.

If we think of the gravity and relative frequency of suppurative affections in individuals poisoned with malaria, we shall experience no surprise that such persons should occasionally suffer from a suppurative inflammation of the middle ear, but all that we know of the pathology of malaria speaks against the theory of a malarial origin of a suppurative process.

We have already noted, in speaking of pneumonia, endocarditis, and various acute inflammations of serous membranes, that, whenever a bacteriological examination has been possible, the ordinary pus cocci or the diplococcus pneumoniæ have been found to be the cause of the inflammation. We do not possess records of any direct researches in this direction in case of purulent otitis media, but there can be hardly any doubt that the same conditions prevail here, and the disease ought therefore to be regarded simply as an accidental complication and not as directly due to the action of the malarial plasmodia.

On the other hand, notwithstanding the absence of anatomico-pathological researches, there is no difficulty in admitting the possibility of labyrinthine hemorrhage (labyrinthitis hæmorrhagica of some writers) when we recall the well-established facts of the pathology of malaria, such as hemorrhage of the retina, of the nerve centres, etc. It is also very probable that degenerative changes in the auditory nerve or its nucleus, or even transitory acute hyperæmia of the internal or middle ear, may be due to the action of the malarial poison.

In conclusion, therefore, we believe that, while there is no reason to deny the malarial origin of certain cases of intermittent otalgia, of

labyrinthine vertigo, of intermittent deafness, or even of intermittent hyperæmia of the membrana tympani, we must nevertheless regard with reserve cases of purulent otitis media of supposed malarial causation.

Olfactory and Gustatory Disturbances.

Cases of anosmia and also of transitory loss of taste have been described as occurring during the malarial attack (Mannaberg), but we have had no personal experience with affections of this character.

Diseases of the Heart and Arteries.

Some authorities (Durosiez, Lancereaux, and others, quoted by Laveran) hold that *endocarditis* may develop under the influence of malaria. This opinion was apparently but erroneously confirmed by the fact that some forms of endocarditis are accompanied by intermittent fever with a protracted course, frequently periodical in its nature, quotidian or tertian in its type, preceded by chills and followed by sweating, with relative well-being during the intervals of apyrexia. The course of this fever in malarial climates leads us to a diagnosis of malaria, but quinine gives no good results, the patient grows worse, cerebral emboli occur, etc. It is unnecessary to recall the fact that when inflammations of the endocardium occur in sufferers from malaria, they must be regarded as intercurrent diseases. We have had cases of ulcerative endocarditis in chronic malaria; in one of these there was a cerebral embolus which caused hemiplegia and aphasia, and brought to an end the large doses of quinine which the physician had been giving for some time under the impression that he was dealing with malarial fever.

What we have said in regard to endocarditis applies also to *chronic arteritis*. Although some French authors, as Lancereaux and Huchard, hold to the malarial origin of this lesion, there are no reliable data to uphold this opinion. In the cases quoted by Lancereaux not only does the malarial etiology fail to appear, but there were no malarial lesions of the liver or spleen. Laveran rightly observes that if the lesions of the aorta and the angina pectoris which they cause were often due to malaria, this causal relationship would certainly not have escaped the observation of physicians practising in malarial regions.

Diseases of the Digestive Apparatus.

We have already mentioned symptoms relating to the stomach and intestines which appear in grave infections. Digestive disturbances as sequelæ in malaria are not frequent, but, on the contrary,

when the attack is over the patients usually have an excellent appetite, and the digestive functions are in good working order, except, of course, in anæmic and cachectic patients. When, however, there are frequent relapses, which are attended by diarrhœas, especially those of a sanguineous nature, the symptom may persist after the attacks and finally present the clinical form of *ulcerative enteritis*. The genesis of this condition is the intestinal localizations of malarial parasites which are repeated in each attack, not so severely as in pernicious malaria, but sufficiently so to subject the epithelium to retrogressive lesions which leave a free field to bacterial invasions, and these result in persistent and even ulcerative enteritis that may end fatally. In this connection it is of interest to recall that in malarial cachexia with amyloid degeneration *gastric ulcers* are found which are due to the malarial condition.

Diseases of the Kidneys.

The kidney lesions in pernicious infections have already been described; they are *intravascular* (parasite-infected red corpuscles, melaniferous and globuliferous phagocytes), *vascular* (lesions of the endothelium), and *extravascular*. These consist in the sometimes extensive necroses of the epithelium, and their exfoliation in the presence of casts and in endocapsular exudations. The latter alterations correspond to those found in other infections, such as scarlatina and diphtheria, which are certainly of toxic origin. In relation to the anatomico-pathological lesions are the functional changes in the kidneys in pernicious fevers (albuminuria with casts, hæmaturia, etc.). From a knowledge of these alterations alone we may infer that in malaria as in other infections there may be acute, subacute, or chronic *nephritis*. Physicians practising in our malarial districts know that nephritis sometimes follows malaria, and in the Roman hospitals we often have occasion to observe cases of this kidney disease in patients who are or who have been affected with malaria.

It is a question whether there is really any connection of cause and effect between malaria and nephritis, or whether it is not merely that the nephritis due to some other cause happens to develop in malarial patients. To form an opinion on the subject we must proceed with great caution, and weigh the arguments justly. Authorities disagree as to the frequency of the kidney affection in malaria, the probability being that this frequency varies in different climates.

We will quote from a few of the writers who have treated of the subject. Rosenstein recognizes an acute nephritis as a result of malarial infection, finding the support for his opinion in the albuminuria which occurs during the febrile attacks. Acute nephritis

(diminished urine, blood, albumin, casts, œdema, etc.) may develop during the course of intermittent quotidian fevers, whatever be the original type. The duration may be of weeks and months, but as a rule both nephritis and fever cease under the administration of quinine. According to Rosenstein, even chronic parenchymatous nephritis may be the result of malaria, and is developed either during the infection, especially if the sweating stage be absent, or at varying periods after the fever has disappeared, even when the patients think themselves sufficiently recovered to return to their work. Bartels believes malarial infection to be one of the most active causes of chronic parenchymatous nephritis. His observations were made in Kiel on patients who came from the plains of Schleswig-Holstein.

Senator, after giving malaria as a cause of nephritis, says that he has seen cases of chronic nephritis in persons who had suffered from malaria, but he does not remember ever to have seen a case in which the infection was the sole cause. Among French authors are to be mentioned Kelsch and Kiener, who have made the subject a matter of special study. They distinguish two anatomico-pathological forms of nephritis:

(1) *Diffuse* or *glomerular nephritis;*

Acute Form.—This occurs in the first period of the infection or during convalescence, and develops in the course of several weeks or months. Anasarca is of considerable amount and of rapid development; the urine is scanty, albuminous, highly colored, sometimes bloody, with abundant sediment, etc. In other cases there is partial and wandering œdema, with clear, abundant, and only slightly albuminous urine; but oliguria may suddenly occur and the urine may contain blood or merely hæmoglobin. With either form the malarial attacks may continue and even be accompanied by icterus. The kidneys are enlarged, the surfaces are smooth, the cortical substance is slightly increased; the glomeruli are voluminous, the pyramids of a dark red color. A microscopical examination shows that the alterations are chiefly in the glomeruli and consist of intraglomerular and periglomerular infiltrations and intracapsular exudations. There are also retrogressive changes in the epithelium, hæmosiderinic pigmentation, hyaline casts, casts containing leucocytes or red corpuscles, etc.

Chronic Form.—The clinical characteristics vary greatly; it may develop insidiously during chronic infection. The kidneys are decreased in size, weight, and consistence; the surface is smooth or finely granular. The principal alteration is sclerosis of the glomeruli, causing progressive sclerosis of the organ.

(2) *Granular nephritis;*

Acute Form.—This develops preferably in persons who have had

many relapses. It begins with rapidly developing anasarca and ascites. The urine is scanty, highly colored, very albuminous, with abundant sediment largely composed of colloid casts. The uræmic symptoms develop with exceptional gravity. The kidneys are large and pale; the cortical substance is increased in size and of a pale yellow color, covered with whitish, rounded and slightly prominent specks; between these granulations the parenchyma is hyperæmic, as are the pyramids of Malpighi; the glomeruli are for the most part anæmic. A microscopical examination shows that the granulations are formed by tubules with voluminous epithelium and dilated lumina; from this epithelium is elaborated the colloid substance which forms the colloid casts. The glomeruli are but slightly altered, a few of them, however, being swollen and of a vitreous aspect. The renal tissue between the specks is hyperæmic; the tubules are narrow and filled with small epithelial cells.

Chronic Form.—This presents the clinical symptoms and the anatomico-pathological alterations of atrophic granular kidney.

From Kelsch and Kiener's description we see that the nephritis of malaria is not distinguished by any anatomical or clinical characteristics, with the exception of its development during or after malarial infection, that serve to differentiate it from nephritis due to any other cause. We might permit ourselves several criticisms upon the description and interpretation given of these forms of nephritis, but it seems of most importance to note simply that possibly amyloid degeneration was not carefully distinguished in these cases, and that several of the forms considered acute were rather subacute or chronic.

Laveran has observed epithelial and interstitial nephritis in malarial patients.

Marchiafava and Ferraresi, after mentioning that they have seen cases of renal alteration in persons who certainly showed the alterations of malarial infection, such as melanotic tumor of the spleen and liver, describe a case of glomerular nephritis in a subject affected by malarial cachexia with hepatosplenic melanosis.

Thayer asserts the frequency of albuminuria in malaria; albumin was found in fully one-half of two hundred and eighty-four cases examined by him and by Hewetson, while acute nephritis was found in four cases. He states that the nephritis following malarial infection is usually an acute diffuse and mild process, similar to that observed in other infections. In some cases the course may be rapid and fatal; in the majority, however, the prognosis is favorable as regards a complete cure. It is not impossible, Hewetson says, that in some cases a chronic diffuse and lethal nephritis should have its origin in malaria,

but definite proofs of this are wanting. Thayer in a recent work, however, states that he has been able to assure himself of the malarial origin of chronic nephritis.

We have observed cases of acute and subacute nephritis developed during grave malarial infection or shortly after its cessation, as occurs after scarlatina. Among others we recall the case of a little girl of seven years who, several days after she was cured of a pernicious infection contracted in the Roman Campagna, had all the symptoms of acute nephritis which lasted several weeks, and then, two attacks of uræmic convulsions having been overcome, made a good recovery.

As to chronic nephritis (large mottled kidney, contracted kidney, granular kidney), several cases have been seen at autopsy in patients with chronic melanotic tumor of the liver and spleen; but as there was nothing to distinguish these renal affections from any other, it can hardly be affirmed without an exact history of the case that they were dependent on the malaria.

Recently Rempicci, of the Roman Medical Clinic, has given us a work upon the kidney lesions in malaria which is without doubt one of the most complete in existence upon the subject. He studied three hundred and fifty cases of malarial infection, and taking into account all forms from simple albuminuria up to true nephritis, those in which the malaria could be asserted to be the cause of the renal lesion as well as those in which this could merely be presumed, there were in all eighty positive cases. By a daily study of the urine of malarial patients at the hospital of Santo Spirito, he became convinced that acute or chronic nephritis may, although infrequently, be developed under the influence of malaria, and the chronic form may either follow the acute or exist as such from the beginning. His cases in which the renal disease was seen from the beginning throughout the course of the malarial infection leave no room for doubt as to the etiology.

The *albuminuria* of malaria, that is to say, the temporary appearance of albumin in the urine, is divided by Rempicci into the *febrile*, which accompanies or follows immediately after the malarial attacks, and the *postmalarial*, which comes after the cessation of the febrile infection or which is observed in cachectics without fever. These albuminurias, especially the latter, are rare. The febrile albuminuria is transitory and very light; the postmalarial, which is frequently accompanied by polyuria, may last somewhat longer; that of the cachectics is also slight, and though it may last for some time, it usually disappears with the improvement of the general conditions.

Acute nephritis either develops during the febrile infection or else follows it (postmalarial nephritis); it is sometimes slight, manifested

only by the œdema, and resolves in a few weeks; at other times it is grave, and presents the symptoms of *acute hemorrhagic nephritis*. If the febrile attacks return during its course, it may become much aggravated and end in a uræmic attack.

Renal complications may occur in every form of malarial infection, the mild as well as the severe. They are more frequent in the last days of autumn and in the winter. Children and young people are more predisposed to them than adults and old people.

The œdema symptomatic of acute nephritis must be distinguished from essential œdema which occurs not infrequently in children and in young persons who have intermittent fever and have become anæmic, without any albumin being found in the urine. Rempicci has observed several of these cases, among others one in a young man in whom there was marked anasarca without albuminuria.

Although the greater number of acute renal attacks which accompany or follow malarial infection end in recovery, there are cases in which the disease passes on to a chronic state; this is more apt to happen when there are later attacks of fever, and when the patient does not follow the hygienic and dietetic regimen prescribed.

Chronic nephritis may develop insidiously, that is to say, without being preceded by the syndrome of acute nephritis. The symptoms are those of chronic parenchymatous nephritis (œdema, abundant albuminuria, many epithelial and granular casts, oligæmia, etc.), nor is there anything which serves to distinguish it from chronic nephritis due to other causes. If fresh malarial attacks occur, there may be an exacerbation of all the symptoms. Rempicci's observations appear to show that chronic nephritis may pass on to contracted kidney. This occurs as in nephritis of other origin, the postscarlatinal, for example, and is marked by disappearance of the œdema, marked increase in arterial tension, hypertrophy of the left heart, polyuria with low specific gravity, scanty albumin, very little sediment, etc. It has not yet been shown whether this form of nephritis can develop insidiously, as it does in the gouty diathesis, arteriosclerosis, etc.

Parenchymatous nephritis may become complicated by amyloid degeneration, especially of the glomeruli, giving true *amyloid nephritis*, or, as it is better to call it, nephritis with amyloid degeneration. We must be careful to distinguish from the renal alterations previously described this *amyloid degeneration* which, although it may involve various organs, finds its chief seat in the kidneys, so that the predominant symptoms are those of changes in the renal functions. To these are added progressive emaciation and stubborn diarrhœa. As in other amyloid degenerations, hypertrophy of the heart is not met with. The urine is abundant, with a low specific gravity, rich in

albumin, but with little sediment. Such amyloid degenerations develop and progress rapidly. A careful clinical examination of several cases shows that a series of estivoautumnal attacks is followed by cachexia with rapid course, in which the symptoms of nephritis are predominant, and that death occurs after uræmic symptoms.

As to the pathogenesis of the renal lesions in malarial infections, we are for the present able only to form theories. We do not as yet know the pathogenic agent of nephritis developing after scarlatina, nor in malaria has the knowledge of the malarial parasite so far thrown any light upon the pathogenesis of the nephritis. In pernicious infections very few parasites are found in the kidneys, while the retrogressive changes in the epithelium may be so grave as to lead to necrosis. From this fact we may infer, as does Bignami, that the lesions are due not to a localization of parasites in the renal capillaries, but to some toxic substance which is brought by the blood to the kidneys and is eliminated by them. In favor of this hypothesis we have the increase in the toxicity of malarial urine recognized by many investigators, and recently also by Rempicci. Of course we can as yet state nothing in regard to the nature of the toxic substances to which the renal alterations are due, whether they are special toxins eliminated by the amœba, or the products of destruction of the parasites, red corpuscles, and phagocytes, or the result of the altered metabolism. And if the toxins are always produced, we still are unable to say why the renal lesions are so rare, or what are the intermediate factors which render the kidneys susceptible.

Glycosuria, Polyuria.—Several observers have noted glycosuria in malaria. Burdel (quoted by Sternberg) says that he found sugar in the urine of eighty out of eighty-six malarial cases. Verneuil (*ibidem*) concludes that malaria often leads to glycosuria, which is transitory when it occurs during the attacks, but which may be permanent when it occurs later. But recent researches do not confirm these opinions. All the investigators who have lately seriously practised uroscopy in malarial infections, such as Rempicci and Massé (quoted by Rempicci), have found no sugar in the many examinations which they have made of the urine of malarial patients. We can recall only one case of acute malarial cachexia from which the patient gradually recovered, but in whom a voracious appetite accompanied by great thirst soon developed, while an examination of the urine, of which about eight litres a day were passed, showed a large quantity of sugar. As the patient was lost to sight, we are unable to state whether this was a case of transitory glycosuria or of true diabetes.

Polyuria in chronic infections is not infrequent. Bignami has collected several reports of polyuria (four to five litres a day) with

slight intermittent albuminuria; the polyuria was manifested after the attacks and had a tendency to increase at their approach.

Diseases of the Liver.

When treating of the pathological anatomy we described the lesions of the liver which are found in chronic malaria, and we saw that the most frequent alteration produced in this organ by the repeated febrile attacks and the ensuing melanosis is a uniform enlargement with increase of the perilobular tissue causing projection of the individual lobules. This hyperplasia of the non-retractile connective tissue, the dilatation of the capillaries, and the hyperplasia of the hepatic tissue are the factors in the increased size, which may be enormous, attaining even to 4 or 5 kgm. (8.8 to 11 pounds).

This morbid sequela in the liver, which was known to the older physicians, usually persists for some time after the infection has gone, even after the disappearance of the melanosis. The alterations do not cause marked disturbances in function of the organ; there is no ascites as in common cirrhosis, no icterus as in hypertrophic biliary cirrhosis, no marked functional disturbances, although a histological examination shows in this *hypertrophic malarial hepatitis* that not infrequently there exists a stasis of leucocytes in the capillary network, from which we must conclude that there was a slowing of the capillary circulation.

When treating of the pathological anatomy we called attention to the atrophies which are found in the liver in progressive postmalarial anæmia, and in old malarial patients who have died of an intercurrent disease, and also to the atrophies consecutive to thrombosis of the portal vein. In cases of pylethrombosis ascites is rapidly formed and reproduced, and there are also intestinal hemorrhages which may be quickly fatal.

A question to which we have already referred is whether malaria can be a direct cause of ordinary cirrhosis, the cirrhosis of Laennec. Upon this point authorities are divided; some, as Kelsch and Kiener and several Italian pathologists, hold that it may be; others, as Colin and Laveran, say that it is a very rare occurrence; and still others, as Frerichs, believe that if cirrhosis develops in malaria, some other cause in addition to the malaria, such as alcoholism, must have exercised an influence on the liver. Our experience leads us to state that although there may be isolated instances of cirrhosis of the liver in old cases of malaria, it is not correct to infer that the malaria is one of the causes of the disease. If that were the case, cirrhosis of the liver would be more frequent in malarial countries than in other

places; which is not confirmed by the observations of Colin, Laveran, Thayer, and ourselves. Moreover, we have seen that an anatomico-pathological study of hypertrophic malarial hepatitis demonstrates that this is a form by itself as to its extension, the seat of the new connective-tissue formation, the capillary dilatation, the lack of compression of the blood and bile vessels, and the relatively well-recognized genesis; it is therefore clearly distinct from atrophic cirrhosis, into which, moreover, malarial hepatitis cannot be directly converted.

Amyloid degeneration of the liver is not infrequently found as the result of chronic malarial infection, or more properly in malarial cachexia.

Diseases of the Spleen.

We have already spoken of the chronic tumor of the spleen. This may lead to several morbid sequelæ. The enlarged spleen, whose weight may often attain several kilograms, sometimes leaves its normal situation, and descending more or less into the abdominal cavity, becomes *movable*. The weight of the organ, the elasticity of the ligaments which hold it in place, and the relaxed condition of the abdominal walls are the principal factors in the dislocation of the spleen, now one and now another predominating; this is the reason why the condition is often found in women, especially in those who have gone through several pregnancies. The new situation of the spleen varies—it may be in the middle of the abdomen, in the left iliac region, or elsewhere, and in any place it may form adhesions and so lose its mobility.

But if the suspensory ligament of the prolapsed spleen becomes stretched or twisted this may cause varices of the veins which empty into the splenic, that is the gastroepiploic, the small gastric veins, etc. We saw a case of this kind in a patient with chronic malaria who died of pneumonia; the large mobile spleen had become displaced, and not only the suspensory ligament was twisted, but also the right portion of the great omentum adhering to it, causing enormous varicosities of the gastroepiploic veins and in part of the veins of the great omentum.

Furthermore, torsion of the suspensory ligament may be such as to compress specially the splenic veins, causing thrombosis in them and consequent necrosis of the spleen. At the autopsy of a woman who had suffered from malarial infection and who died of influenza, the spleen was found to be fixed in the right iliac fossa, adherent to the cæcum and to the uterine appendages of the right side; the organ which had travelled so far had old thromboses of the blood-vessels, was enlarged to twice its original size, and was converted into a homogenous mass of a dark greenish-gray color and oily to the touch. A microscopical examination showed that the splenic tissue had become

a mere granular detritus containing crystals of cholesterin and numerous flakes of bilirubin acicular crystals.

The ectopia and mobility of the spleen often occasion pressure pains in the abdomen, especially during walking—pains which may radiate to distant portions of the body, even to the ribs and the left shoulder. Other effects of the stretching are vomiting, and even symptoms of intestinal occlusion if the spleen compresses any part of the intestinal tract, such as the sigmoid flexure.

A fortunately rare but very grave accident is *rupture of the spleen* of which we read in the literature of all malarial countries. Tension of the capsule and a softened parenchyma are the anatomical conditions favoring this rupture in cases of recent enlargement, while thickening and partial but tenacious adhesions of an enormously enlarged spleen, causing unequal resistance of the capsule in its various portions, facilitate rupture in chronic infections. Rupture sometimes occurs from falls or from blows upon the abdomen, and this gives rise to frequent medicolegal discussions; but it may happen spontaneously or from the natural movements of the body, such as straining in defecation or vomiting, as has been proved several times in the hospitals of Rome. Cimbali observed rupture of the spleen in the hospital of Santo Spirito, in the case of an old man, a malarial cachectic, who simply got out of bed. When the rupture is due to traumatism, as a fall from a height, we find many cracks in the splenic capsule, giving issue to blood. When the rupture takes place spontaneously, the mechanism is different; one or several hemorrhages occur in a portion of the interior of the parenchyma, the blood burrowing under the capsule finally detaches it to some extent, it becomes lacerated at the point of least resistance, and then the blood pours out into the peritoneal cavity. In these cases, at the autopsy, the external laceration of the capsule is found to lead into a large or small cavity in the parenchyma of the spleen, which contains fluid or coagulated blood.

In malarial infection there are sometimes central hemorrhages in the splenic pulp without rupture.

In the great majority of cases rupture of the spleen is manifested by sharp pain in the left hypochondrium, symptoms of internal hemorrhage, and collapse in which death occurs. In the case observed by Cimbali, the patient first had the pain in the left hypochondrium, and then became cold, pale, and cyanotic; the pulse was rapid, the breathing was difficult, superficial, or stertorous; there was vomiting; death occurred during sopor about an hour after the appearance of the first symptoms.

But several hours or even one or two days may elapse between the initial pain of the laceration and the fatal issue. A case observed in the hospital of Santo Spirito by Bignami was that of a young man

suffering from a relapse of estivoautumnal fever, who had a prominent enlargement of the spleen; suddenly he felt a sharp pain in the left hypochondrium which spread over the whole abdomen and was increased by pressure; gradually his strength gave way, the skin became pallid, the pulse small, there was vomiting, and after about forty-eight hours of suffering death occurred in collapse.

At the autopsy was found a not very extensive laceration of the capsule of the spleen, from which there led a canal, formed by the dissection of the capsule, which ended in a large effusion of blood in the parenchyma. In the peritoneum there was an abundant amount of fluid and coagulated blood.

Bastianelli observed two important cases of rupture of the spleen during acute pernicious infection, the symptoms of which became confused with those of the rupture. In the first case the patient was brought to the hospital in a state of coma in which he died twenty-four hours later. At the autopsy it was found that the peritoneum contained a large amount of blood, and that a large clot was situated above the convex surface of the spleen. In the brain there was slight melanosis; the liver was melanotic. A microscopical examination of the viscera revealed the parasites of pernicious fever. The spleen was enlarged to four times its usual size, the capsule was detached from it by a large quantity of coagulated blood which extended over the whole surface of the organ. The capsule was not widely ruptured, but at the point of entrance of an artery of medium calibre, which penetrated into the spleen from its convex surface, there was a small fissure upon which was situated the external coagulum. Upon section of the spleen there was found a central mass of blood from which a path led up to the capsule, and along this path the blood had travelled to do its work of dissection. The spleen was soft and melanotic.

In the second case also the patient was brought to the hospital in coma, and died after a few hours, the diagnosis, made from an examination of the blood, being that of grave malarial infection. At the autopsy, there were found melanosis of all the organs, abundant hemorrhage into the peritoneal cavity, and upon the convex surface of the enlarged spleen a rupture about 2 cm. (four-fifths inch) long. Upon section it was found that the laceration corresponded to a hemorrhagic area of triangular form, situated beneath the capsule, with its apex towards the hilum, its base at the periphery. In the melanotic splenic pulp were several other small hemorrhages, some in the substance of the organ, and some subcapsular. An examination of a fresh specimen of the pulp of the spleen and of other organs showed parasites in various stages of development, many crescents, and a large number of pigmented leucocytes.

The time elapsing between the laceration and death depends chiefly upon the extent of the tear. If it be large, then in a very short time a great amount of blood is effused into the peritoneum, but if small then the blood flows slowly until in a few hours a degree of acute anæmia incompatible with life is reached. The direction of the laceration must also have some influence upon the amount of blood which escapes in a given time. These facts are worth knowing in order that the physician may understand how to guide himself in regard to surgical intervention in individual cases of rupture of the spleen.

Some authors, as Laveran, speak of malarial *abscesses of the spleen*. These are evidently the result of pyogenic infection. We recall a case in an old malarial patient who died from hydræmia accompanied in the last days of life by attacks of fever preceded by chills and followed by profuse sweating. At the autopsy two enormous abscesses were found in the greatly enlarged spleen, in the centre of each of which was a discolored and almost completely desiccated hemorrhagic infarct; in the liver were numerous small abscesses some of which were confluent. An examination of the pulmonary veins, of the left heart, the aorta, and the splenic artery failed to reveal any lesions which might prove an embolic origin of the splenic infarcts.

The Blood.

Leukæmia.—Nearly all works on pathology state that leukæmia sometimes occurs as a result of malarial infection, although none of them gives satisfactory data to sustain the affirmation. Mosler reports that from the statistics of one hundred and twenty-four cases of leukæmia he finds that only eight or ten were unquestionably developed after intermittent fevers, and then only when these were persistent, irregular, and of long duration. Our own experiences show the extreme rarity of leukæmia following chronic malarial infection. We can recall only one case in which the relation between the two diseases seemed to us to be probable. It was that of a countryman, twenty-eight years old, who for many years had been in the habit of going to the Roman Campagna in the summers, where he several times contracted the fever which was always followed by several relapses. In the last year (1879) he was again taken with fever and came to the hospital with enormous enlargement of the spleen and liver, diarrhœa, and grave anæmia, and died two weeks later, the diagnosis being that of malarial cachexia. The anatomico-pathological examination showed advanced splenomedullary leukæmia, and, what is worthy of note, in the liver as well as in the spleen was found

black pigmentation, which in the first of these organs was evidently perilobular. Bastianelli observed another case similar to this, even to the splenohepatic melanosis. In Mosler's cases, as well as in those seen in Rome, the leukæmia was splenomedullary, and there were no enlargements of the lymphatic glands. It is evident that we cannot assert positively that chronic malaria, which is itself not accompanied by an increase in the number of leucocytes, is the cause of leukæmia; some other factor as yet unknown must be interposed between the two diseases.

Pernicious Anæmia.—We have already discussed anæmia in both acute and chronic malaria, its varying degree according to the nature of the infection, its etiology, and its genesis. Here we desire to speak of the pernicious anæmias which follow the anæmia so closely connected with malaria. As we know, the anæmia of malaria is not always rapidly and completely cured. Although prompt and entire recovery from the morphological blood crasis frequently occurs in mild infections, the same cannot be said of the estivoautumnal infections. Even when the malaria is overcome an amount of anæmia usually persists which is often recognized by an objective examination alone; in light cases we may have to count the red corpuscles and determine the amount of hæmoglobin present. Now this malarial anæmia, the direct result of the infection, may be followed by anæmic states differing in gravity, duration, and result; these anæmic conditions become autonomous, because they continue after the disappearance of the infection. They may occur after both the acute and the chronic form of the disease; in the case of the acute they come when the affection has lasted several days without receiving treatment, and when the number of parasites is sufficiently large, or when to the ravages of the parasites are superadded hemorrhages, as in hemorrhagic pernicious fevers. In the chronic form they come when relapses are frequent, the digestive functions impaired, and when the hygienic conditions surrounding the patient are bad.

According to the researches of Bignami and Bastianelli and Dionisi we may distinguish several types in these postmalarial secondary anæmias, which have a different clinical course and different appearances in the blood.

The ordinary malarial anæmia, instead of being followed by a progressive though slow improvement in the condition of the blood, is succeeded by an anæmic state which is protracted and aggravated independently of any return of the infection. Its duration may be weeks or months, the subjective and the objective symptoms being those of ordinary anæmia. When with the anæmia there is a marked splenic enlargement, with no parasites in the blood, but evidences of a

secondary anæmia, especially if there are irregular elevations of temperature, we are led to think of what is called splenic anæmia. An examination of the blood shows alterations similar to those of secondary anæmia; that is to say, a diminution of the red corpuscles, nucleated normoblastic red cells, rarely any megaloblasts; yet no leucocytosis, but rather a diminution of leucocytes with a relative increase of the mononucleated cells. This is the first type.

In other cases, fortunately very rare, the malarial anæmia is succeeded by true pernicious anæmia. Debilitated patients, women, and the aged are especially predisposed to this form of the affection. Pregnancy, which in itself creates a predisposition to pernicious anæmia, favors its development, as shown by many cases. The clinical symptoms and the anatomico-pathological data are the well-known ones of pernicious anæmia; even the bone marrow shows the lesion long recognized, and because of its peculiar characteristics called by Ehrlich "megaloblastic degeneration." An examination of the blood shows in addition to the marked diminution of red corpuscles, poikilocytes, microcytes, red corpuscles of normal size or larger than normal, which stain differently from the normal red corpuscles, and nucleated red corpuscles in large numbers, chiefly megaloblasts. This pernicious anæmia is not directly secondary to malaria, but follows it as it does other infective diseases, by intermediate steps as yet entirely unknown to us. This fact is in harmony with the most recent hæmatological researches, from which it appears that the return of the bone marrow to the embryonal type, which characterizes the megoblastic degenerations of pernicious anæmia, is not found in secondary anæmias. Moreover, the experimental studies of Bignami and Dionisi in chronic anæmia due to toxic hæmatolytic agents (pyrodine) show that however advanced may be the anæmia, no megaloblastic degeneration is produced in the bone marrow, nor are gigantoblasts found in the blood.

In a third form of anæmia, which is also progressive and pernicious, we have the clinical course of pernicious anæmia, in this case somewhat rapid; but an examination of the blood does not show any nucleated red corpuscles. The anatomico-pathological examination explains this, as the marrow of the long bones is quite yellow, and even that of the short and of the flat bones is poor in hæmatoblasts to such an extent that not only is there no new formation of hæmatoblastic marrow, but the normally functioning marrow of adults diminishes its work. In this type of postmalarial pernicious anæmia (resembling a case of post-hemorrhagic secondary anæmia reported by Ehrlich which became progressive from lack of compensation on the part of the bone marrow) accurate observations of the blood dur-

ing life are lacking, but Bignami has studied two cases anatomico-pathologically.

Malarial Cachexia.

This is the most advanced stage of the physical deterioration produced by chronic infections. When these have been protracted, when the patient continues to live in a malarial region, and when the hygienic conditions of his surroundings are bad, he falls into a state of cachexia, which differs from that of other diseases (carcinoma, diabetes, Addison's disease) in that it is not absolutely lethal, and that it may be overcome. Many examples of this cachexia are seen in the country and in malarial regions, which are easily recognized even by the laity by the earthy sallowness of the skin, the emaciation, the swollen abdomen, and the languid look and walk of the patients. No age is spared by cachexia; men are more subject to it than women, because more exposed to the infection in the discharge of their daily work. In some elevated places which are exempt from the fever, below which are situated plains which are fertile but ravaged by malaria, the contrast between the women who do not go down to the plains to work and the men who pass the greater part of their lives in field work is most striking.

Grave anæmia, enlargement of the spleen and liver which is sometimes enormous, emaciation, lack of appetite, dyspepsia, diarrhœa, and frequent hemorrhages from the nose and the gums are the chief symptoms of the cachexia, and these account for the earthy pallor, the weakness, apathy, torpidity, difficulty in moving, and nervous disturbances. Everything shows that the prolonged infection has almost exhausted the resources of the organism and has worn out its compensatory powers, so that life is maintained against a thousand obstacles, with a balance of strength scarcely equal to the demand.

The grave anæmia is immediately apparent on external examination alone; the examination of the blood shows notable diminution in the red blood cells, which may be less than a million; a few nucleated red cells, normoblasts, are found when the bone marrow compensates just enough to prevent aggravation of the anæmic condition and maintains it in a stationary condition. In cases of very grave and progressive cachexia the nucleated red cells are absent, and at the autopsy the bone marrow is found to be yellow, sclerotic, or gelatinous, with no or with very few, nucleated red cells. The malarial parasites are seen if the infection is actually present; otherwise they are either absent altogether or else only the crescent forms are present. Sometimes these patients have an elevation of temperature without any malarial parasites being found in the blood, or without any other

infection being discoverable to account for the fever. The cachectic state may go on for months and years, with alternating improvement and aggravation, the first in the winter and the second in the summer when the patient is exposed to new infection.

It is an interesting fact that even when these cachectics are exposed to fresh infection in the season of grave malaria, so far as we know they do not have pernicious malaria. It has never been our experience to observe in cachectics grave infections with many parasites in the blood, but always fevers with few parasites and very little melanæmia. If the patient does not change his place of abode, after a struggle he dies of exhaustion or more often of some intercurrent disease.

Kelsch and Kiener distinguish in chronic malaria: *cachexia with a surplus of iron in the organs* (spleen, liver, bone marrow, kidneys), and *cachexia with atrophy of the organs*. The first should be regarded as a postmalarial pernicious anæmia; the opinion of Kelsch and Kiener that this morbid condition is the direct result of the action of the parasites, which do not produce black pigment, whence the absence of melanæmia and melanosis, but only destroy the red corpuscles, whence the hæmosiderosis, does not receive support from the data furnished by recent researches, but on the contrary is proved to be untenable. The second is merely the terminal stage of some forms of cachexia in which atrophy of the liver is found; it is either primary or is secondary to thrombosis of the vena portæ; it causes the abundant ascites and sometimes rapidly lethal gastrointestinal hemorrhages.

Cachectics are predisposed to many infection diseases, such as pneumonia, ulcerative endocarditis, dysentery, erysipelas, and septicæmia, which may follow even slight external lesions—the more severe lesions perhaps giving rise to phlegmon and gangrene. In the cases of hydræmic cachexia with gangrene reported by Kelsch and Kiener there was no active malaria, nor had there been any very recently, as was proved by the absence of melanosis in the hæmatopoietic organs.

From this cachexia which represents the last stage of an infection, or more properly of a long protracted succession of infections, we must distinguish those cases in which the symptoms of cachexia are rapidly manifested after a grave malarial infection which has lasted only a few weeks or months. Marked anæmia, œdema, general prostration, weakness of the heart, invincible apathy, hemorrhages, dyspepsia, diarrhœa, and albuminuria are the most frequent symptoms. According to Kelsch and Kiener, anasarca is often seen in Algeria in cases of acute cachexia, and even seems to be "epidemic" among cachectics, while gangrenous phlegmons, bedsores, and gangrene in various parts of the body often occur.

Acute cachexia is most frequently seen in persons who have come from healthy regions to malarial ones, where they catch the infection and continue to reside. Thus in Sicily Tomaselli has observed that the persons most subject to acute cachexia are workmen who come from the regions in Northern Italy which are non-malarial. After a few attacks of malarial fever the symptoms of grave cachexia supervene, namely, loss of strength, mental depression, a color between pale and earthy, emaciation, œdema, dyspepsia, diarrhœa, icterus, bleeding gums, splenic enlargement, etc.

Patients may recover from malarial cachexia, provided that the fever stops, that they leave the infected region, and that the digestive functions are restored. This improvement is sometimes seen in the hospitals. We recall the case of a youth who returned from the Congo in a condition of advanced cachexia, with enormous splenohepatomegaly; after a few months' sojourn in a healthy region he became so well and so vigorous as to be scarcely recognizable; the liver had returned to its normal size, and the spleen, which had occupied a good part of the left side of the abdomen, was only slightly prominent during inspiration.

Acute or chronic malaria may, however, be followed by amyloid degeneration. Then the course of the cachexia becomes rapid; symptoms of nephritis are superadded, because, as we have seen, the kidneys are the chief seat of the amyloid degeneration; the patients become profoundly anæmic, and have anasarca, diarrhœa which yields to no treatment, and finally die with uræmic symptoms. An examination shows neither nucleated red corpuscles nor in some cases eosinophile cells. At the autopsy we find parenchymatous nephritis with amyloid degeneration of the glomerules, arterioles, and even the walls of the uriniferous tubules; and also degeneration of the digestive tract, the spleen, and the liver in which the process is, however, usually in an incipient stage. In these cases it is not unusual to find numerous simple ulcers of the stomach, with the arterioles and capillaries in a state of amyloid degeneration. This condition is, however, not constant in malarial cachexia, so that predisposition is necessary to its production, and this is probably the case in other diseases in which amyloid degeneration occurs.

COMPLICATIONS.

The question of complications in malarial infections was the subject of investigation and discussion even before the discovery of the parasite, the most divergent opinions having been emitted, some of which were not only erroneous but even harmful to practice. The

greatest error of all, as we know at present, was the teaching that some of the complications of which we shall speak were produced under the direct influence of the malarial agent.

We will briefly review the subject of these complications, in order to note the special course of their symptoms when they are developed in organisms in which the malarial affection is in active operation, or from which it has but recently disappeared, or in which it has left marked alterations, such as we find in postmalarial anæmia and cachexia.

Pneumonia is the disease which has provoked the greatest discussion, and whose etiology and genesis in malaria have caused more divergence of opinion among practitioners than any other complication, with resulting differences as to the theories of treatment.

Is there a pneumonia caused by malaria? Upon this point the discussion may be considered to be closed, as we are able to assert that malarial infection *per se* is not capable of producing inflammation of the lungs—a fact which Baccelli long ago had in mind when he stated that malaria had no phlogogenic power. If, therefore, pneumonia develop in subjects suffering from malaria, with parasites in the blood, it must be regarded merely as a morbid accompaniment of the infection; indeed, a bacteriological examination of the inflamed lung shows the presence of pneumococci, a fact which has frequently been demonstrated since we first called attention to it (Guarnieri, Ascoli, Nazari, and others). The association of the two infections can be recognized by experienced practitioners, when in the course of a pneumonia a malarial first invasion or relapse causes an exacerbation of the temperature, preceded by a chill and followed by sweating. But in order to diagnose positively the malaria an examination of the blood must give unmistakable results, and we must be able to exclude a further extension of the pneumonia or endocardial complication. On the other hand, pneumonia may be insidiously developed during a grave malarial infection, or a prolonged or subcontinuous complicated pernicious infection, and because of the greater prominence of the malarial symptoms be discovered only on physical examination or at the autopsy. If energetic treatment succeed in overcoming the malarial symptoms, then those of pneumonia take their place, sometimes with a grave course and delayed crisis preceded by pseudocrisis.

We frequently find areas of pneumonia, which are sometimes bilateral, in patients who have died of a malarial affection which for several days has been grave in its course. Histological examination of sections of lung shows parasite-infected red corpuscles and melaniferous phagocytes within the capillaries, while the diplococci of

pneumonia are found in the lumen of the alveoli in the midst of the exudate, where only normal red blood corpuscles and polynuclear leucocytes without pigment exist.

Pneumonia may also occur during the course of a mild tertian or quartan infection; when the malaria has been overcome with quinine the pneumonia continues its clinical evolution with regular and prompt crises. This fact was reported in the writings of the older physicians, but is even more clearly demonstrated in recent works, among which those of Antolisei and Ascoli of the Medical Clinic in Rome are prominent.

We have already called attention to *pneumonic pernicious fever* in which, during the febrile attack, we note thoracic symptoms, such as dyspnœa, cough, and bloody expectoration, and in some portion of the chest (usually in the posterior-inferior part of one side) we get dulness, fine crepitant râles, and even bronchial breathing. These are symptoms which, although due to congestion of the lungs and a sero-sanguinolent exudation, disappear with the disappearance of the fever, but may return if there are fresh attacks. As we have already stated, cases of pneumonic intermittent fever were observed by Baccelli and others before the discovery of the parasite, but none has been reported with positive results from examination of the blood. In any case pneumonic intermittent fever should be distinguished from pneumonia with intermittent fever. Basing their doubts upon observation of the course of the fever in malarial climates, physicians have been inclined to doubt the coexistence of pneumonia and a malarial infection, but in the present state of our knowledge the doubt can be eliminated. Several cases of pneumonia with intermittent fever are reported in medical literature, and there are frequent occasions to observe them in practice. Ascoli has recently noted a case of acute fibrinous pneumonia, during the course of which there were six quotidian febrile attacks, in two of which the temperature rose to 41° C. (105.8° F.), and in one it went still higher. The attacks began with a severe chill and ended with profuse sweating in complete apyrexia; there was also enlargement of the spleen. But examination of the blood gave only negative results; quinine was not given; the crisis came as usual on the seventh day, and recovery was perfect.

A *pneumonic intermittent fever* has also been described, in which after a few attacks the pneumonic symptoms become continuous as in true pneumonia, and the fever also becomes a continued one. All the symptoms point to a true pneumonia, but if malaria is held responsible for the first attacks, we shall have to admit that the pathogenic organisms of fibrinous pneumonia must have found some way

to flourish in the areas congested as a result of malarial vasomotor paralysis.

It is well known to all physicians practising in malarial regions that in the winter and spring epidemics of pneumonia, the disease especially attacks the poor field workers who are every now and then subject to relapses of malaria contracted in the summer and in the autumn, and even more does it attack the anæmic and cachectic. The clinical course is as a rule more grave than in healthy subjects; a typhoid state with cerebral symptoms is often promptly made manifest. Diarrhœa, vomiting, icterus, albuminuria, bloody or yellow sputum, are the ordinary symptoms of this pneumonia in patients already enfeebled by anæmia, fever, poor food, and the prolonged and arduous labors which necessity obliges them to perform.

The lethal ending may occur in two or three days from the beginning of the disease, or sometimes after unexpected aggravation of the symptoms. Recovery, when it takes place, is slow.

The mortality is high (sixty to seventy-eight per cent. according to Ascoli) in the pneumonia which attacks those who have long been malarial subjects. If they escape pernicious fever they succumb to the pneumonia. This is the gloomy prognosis in chronic malarial patients, and especially in cachectics, of whom there is a veritable slaughter by pneumonia in winter.

The autopsy shows that in gravely anæmic and cachectic malarial patients the pneumonia, as Cantani noted, is asthenic; that is to say, there is an exudate scanty in fibrin and chiefly serous, with abundant epithelial desquamation. There is also a pneumonia which is not really specific in malaria but when terminal is also met with in other cachexias, especially that of nephritis, and which is the result not of any special agent, but of the condition of the organism in which it develops.

Other peculiarities of the pneumonia of malarial patients worthy of note are the frequent occurrence of diplococcic septicæmia, delayed resolution, and subsequent induration. Recent researches upon the subject of pneumonia in patients exhausted by prolonged malaria, anæmic, and with enlarged spleen and liver, have demonstrated the frequency of *pneumonic septicæmia*. Bignami describes it in four malarial patients in whom there were also found pneumococci in those portions of the subcutaneous tissue where stimulating injections had been made. Nazari confirms this fact. In sixteen cases studied by him in the winter, he found diplococcic septicæmia in four, three of which were cases of malarial anæmic patients. Such septicæmias always occurred in estivoautumnal malaria; pneumonia coexisting with mild malaria and accompanied by septicæmia has so far not been observed. Septicæmia, a result of diminished resistance of the

blood to the dyscrasia, is necessarily a factor of the gravity of pneumonia in chronic malarial patients. To the same cause are due the frequent complications, especially ulcerating endocarditis, meningitis, and nephritis.

Delayed resolution in pneumonia complicating malaria is frequently noticed in hospitals in malarial regions. After the crisis, the physical signs of hepatization persist, and gradually resolve, often without the râle redux, sometimes with evening rise of temperature which causes a suspicion of metapneumonic or tuberculous collections.

Induration of the hepatized portions of the lung by the neoformation of connective tissue between the alveoli, the infundibula, and the bronchioles is not infrequently seen in our hospitals, to which flock the malarial patients affected with pneumonia. The history of the patients and the physical examination show advanced malaria, and at the autopsy we find the characteristic changes in liver, spleen, and bone marrow. This result of pneumonia may be diagnosed by the persistence of the physical symptoms of hepatization. For several days after defervescence has occurred the patient has slight fever in the evening, causing, as in the case of delayed resolution, a suspicion of metapneumonic exudations or of tuberculosis; but although the fever may stop after several days or even weeks, the signs of total or partial induration of one pulmonary lobe persist, and there is sinking of the thorax corresponding to the sclerosed lobe. In time this condition may cause bronchiectasis with all its special consequences.

Induration does not always occur in the manner just described; not infrequently the cases are further complicated by endocarditis of the left and even of the right heart. The affection may run on for two or three weeks with intermittent fever due to the endocarditis, and a fatal issue is often hastened by a purulent embolic meningitis due to diplococci, as is the meningitis developed at the height of a pneumonia; it differs from it, however, in not being diffuse, but in being confined to small areas at the convexity and at the base.

Postpneumonic pulmonary induration, ulcerative endocarditis, and embolic meningitis constitute a triad not infrequently seen in the subjects of a long-standing malarial infection. Cases of primary and of metapneumonic pleurisy have been described, during which there occurred attacks of intermittent malaria.

We have mentioned the *septicæmia* which occurs during the course of the pneumonia. When chronic malarial infection is accompanied by anæmia, enfeeblement, and especially by cachexia, the ground is found to be favorable to the development of other septicæmias, especially the pyogenic. Bignami has studied two cases of septicæmia in old malarial patients, one characterized by the presence of

the staphylococcus aureus, truly cryptogenic; the other by that of the streptococcus in which the symptoms of septicæmia were preceded by tonsillar angina.

Erysipelas in the subjects of malarial cachexia is often followed by streptococcic septicæmia. Guarnieri and Bignami have seen two cases. In Bignami's case, the patient was an old man who had become profoundly anæmic after many febrile attacks, one of them of pernicious fever. One day he was found with œdematous infiltration of the face and neck, slight fever, and sopor; the urine contained no albumin; after a few days the patient died. At the autopsy there were found, in addition to the signs of a recent malaria, an enormous streptococcic invasion of the infiltrated cutis, and many streptococci in the pulp and veins of the spleen. On careful examination of the skin of the face there was found a small cutaneous wound at the left border of the inferior maxilla, which had been made by a barber the day before the appearance of the œdema.

Typhoid fever has been regarded by many as a frequent complication of malaria, but we have not seen one case of coexistence of the two affections, nor have Baccelli and many others seen any, either before or since the discovery of the parasite of malaria. We do not mean to say that in regions where the existing conditions are favorable to the development of the two affections, as sometimes in the Roman Campagna, they may not coexist; that is to say, that during the course of a typhoid fever a malarial attack may occur as a primary invasion or as a relapse. The antagonism believed in by Boudin (quoted by Laveran) has not been substantiated—indeed, the contrary is proved by cases of the coexistence of the two infections reported by Thayer and Barker, and published by Osler and by Gilman Thompson. But that from the union of these two diseases we may have a third morbid form, called typhomalaria, although this opinion has been repeatedly advanced by writers, commencing with Woodward (see Sternberg), who was the first to introduce the name, is not only open to doubt, but everything seems to prove that it does not exist. Since examination of the blood came into use as an assistance to the diagnosis, many morbid forms that were supposed to belong to typhoid malaria are now known to be due to the typhoid infection, to the diagnosis of which assistance is also derived from bacteriology (Widal's reaction). And what fearful abuse was made of quinine in this disease, when the too vivid imagination of the physician conjured up an insidious and strangely rebellious malarial infection, an abuse which did not even cease with symptoms of poisoning! Even the criterion of the older physicians—*adjuvantibus et lædentibus*—was forgotten in these cases! That which led to the belief in the union

of typhoid fever and malaria was the intermittent fever which followed the course of the continuous fever of typhoid, especially when it was preceded by chill and ended in sweating. But we now know that these fevers are typhoidal or posttyphoidal, and that malaria has nothing to do with them. As to the meaning of the term *subcontinuous typhoid*, of Baccelli, we have already spoken. By it is meant a grave malarial infection, with intermittent fever, accompanied by a typhoid state which lasts a few days, and which may simulate grave typhoid fever. Subcontinuous typhoid has been called *remittent typhoid* (Kelsch and Kiener), *continuous malarial fever with a typhoid state* (Laveran), and *typhoid malarial fever* (Squire). The observations of Laveran, Kelsch and Kiener, Ascoli, and others, have shown that typhoid fever may be developed shortly after the cessation of an attack of malarial fever. If death occurs, we find in addition to the typhoid lesions in the ileum, etc., melanosis of the liver, spleen, and bone marrow. Kelsch and Kiener interpreted these conditions as proofs of the existence of typhomalarial fever. But it is now known that melanosis alone does not indicate active malaria, nor does swelling of Peyer's patches and the mesenteric glands without other signs in cases of pernicious malaria indicate coexistent typhoid infection.

Finally we may add that after grave malarial infection, there sometimes occurs an intermittent or remittent fever not accompanied by grave symptoms, lasting one or even two weeks, which is certainly not malarial as proved by the negative results obtained by an examination of the blood. Since no symptom authorizes us to regard this as typhoidal in its nature, or due to any other special infection, we have proposed calling conditions of this sort *postmalarial fever*.

In those hot countries where both *dysentery* and malaria are found, it is not unusual to see the two diseases coexisting (Laveran). Even in temperate climates, in the summer and autumn, dysentery may be seen united to malarial fever; of course in this we do not include those frequent cases in which mucosanguineous dejections occur only during grave febrile attacks. Thayer describes cases of the association of the two affections, and regards them as examples of the association of two infections from protozoa. At this date it is unnecessary even to recall the heated discussions held between some writers as to whether malaria *per se* could be the cause of true dysentery; it is now known beyond a doubt that we have to do with two different diseases, of different etiology, which may flourish contemporaneously in the same district.

It will easily be understood that the union of the two affections would be very harmful. When dysentery appears in a person weakened and rendered anæmic by malaria, the bloody discharges, pain,

fever, dyspepsia, etc., must reduce the patient to a very serious condition. And if one suffering from dysentery is attacked by estivo-autumnal malaria, the latter may become pernicious from the individual conditions present. Of the two patients observed by Laveran, one died in collapse; in the other the attack was a choleraic pernicious fever which was cured by quinine. A clear example of parasitic localization in the place of least resistance!

Cholera may be associated with malarial infection. During the last light epidemic of cholera which occurred in a summer when grave malarial infection was also very active, a case of the association of these two affections was observed by Bastianelli. An examination of the blood showed the presence of malarial parasites, and a bacteriological examination of the dejecta showed the cholera bacillus. Sternberg also reports cases of this association, and expresses the opinion that it always occurs in choleraic pernicious fever. It is scarcely necessary to state that this theory does not correspond with the long series of facts which are well known to us.

That other infections can be associated with malaria is proved by the fact that we have seen the malarial infection coexisting with *smallpox*, that Laveran has seen two cases of smallpox following malaria, and Antolisei noted intermittent malarial fever in patients convalescing from smallpox.

Among the chronic infections which in their relation to malaria deserve the greatest attention, is *tuberculosis*. Some French physicians practising in Algeria (Boudin and others) have held that there is a certain antagonism between tuberculous and malarial infections, basing their opinion chiefly on the fact that tuberculosis is rare in Algeria where malaria is endemic. But even though it is true that the disease is rare in Algeria and in other hot countries where malaria is common, we cannot conclude that there is antagonism, because there are countries where both obtain, as India, Bengal, Tonquin, the West Indies, and Guiana, where next to malaria, tuberculosis is the most frequent disease (Laveran). On the eastern coast of the United States, tuberculosis and malaria occur with equal frequency (Thayer). The same can be said of the malarial regions around Rome; indeed the experiences which we have had in the Roman hospitals enables us to formulate the following propositions:

(*a*) Active tuberculosis, even when very extensive, does not exclude even fatal malaria. There are many well-known cases of tuberculous patients who, because their disease was neither extensive nor of a grave nature, have been allowed to go on with their work, and who have contracted malarial infection; and when this was overcome the tuberculosis continued its course. But we also know of cases of fatal

pernicious malaria occurring in tuberculous subjects. Among others the following is of interest:

A young wagoner, 25 years of age, was brought in a comatose condition to the hospital, where he died the same day. In the blood were found small amœboid parasites, pigmented leucocytes, nucleated red cells, and parasite-infected and melaniferous endothelium. At the autopsy, in addition to the lesions of acute grave malaria (very large, soft, and melanotic spleen, melanosis of the liver, etc.), the right lung was found to be adherent to the thoracic walls and to the diaphragm by a soft connective tissue very rich in miliary tubercles and permeated by both large and small cheesy masses, while in the interior of the lung there were numerous areas of tuberculous bronchopneumonia, and one large cheesy mass conical in shape, with its apex terminating in a small bronchus and surrounded by a thick zone of miliary tubercles. The peribronchial glands were also tuberculous. A microscopical section of the pleura and lungs stained according to Weigert's method showed the tubercle bacilli in the tuberculous tissues, and the amœbæ of malaria in the blood-vessels.

(*b*) If the malarial infection attacks organisms affected by tuberculosis, the latter is not arrested, but acquires a tendency to spread, and produces miliary tuberculosis. We are led to this proposition by the observation of cases in which was found miliary tuberculosis, preceded by tuberculosis of the lymphatic glands, with either active or recently spent malaria. In one of these cases the blackened spleen was dotted all over with tubercles. A case recently seen in the hospital of Santo Spirito is of importance in this connection; it was that of a child suffering from meningeal symptoms, which were attributed to malaria (meningeal pernicious fever), because numerous estivoautumnal parasites were found in the blood. The autopsy showed a tuberculous meningitis, which had been preceded by tuberculosis of the peribronchial glands and active malaria, as had already been demonstrated by an examination of the blood, and as was also shown by the much softened and black splenic tumor, rich in malarial parasites; the latter were also found, though in small number, in the minute vessels of the brain.

That those who have suffered much from malaria and cachectics easily contract tuberculosis is a thing which we cannot affirm. On the contrary, according to our experience, malarial cachexia does not predispose to tuberculosis as do other cachexias, for instance that of diabetes.

Other diseases, which we have not taken into consideration, may complicate malaria; such are the exanthemata and puerperal and surgical infections.

In the study of the *puerperium* in relation to malaria, it should be

remembered that pregnant women who are suffering from malaria are predisposed to grave and even to pernicious anæmia, as we have already mentioned when discussing postmalarial anæmia, and to abortion. From the statistics of abortion in malarial countries, it is shown that this occurs more frequently than in non-malarial countries. It is easy to understand how on the one hand the anæmic condition predisposes to hemorrhages and to puerperal infection, and on the other that, if the malarial infection still persist, the puerperium should occasionally excite to new attacks of fever. Hence the danger that the fever of puerperal infection should be mistaken for malaria, and *vice versa*. The first error, especially before the discovery of the parasite, was the more frequent and led to the useless and often injurious administration of quinine in doses which were sometimes enormous, and, worse yet, to delay in the effort to find the source of the infection.

The same may be said of *surgical fever* following traumatism and operations. Latent malaria is often roused into activity by wounds and by surgical operations, whence the necessity of examining the blood whenever the origin of the fever is in doubt. Thayer reports a case of pernicious fever occurring after removal of cancer of the tongue, and cured by large doses of quinine given intravenously. But chronic malaria may react upon the wound, causing difficulty in the process of cicatrization, and facilitating pyogenous infection.

Insolation was mentioned in the section on pernicious fevers, and the conclusion was reached that its occurrence accounted for those cases of disease which occurred in the heated term in men working in the country, and which had a rapidly fatal course, with nervous symptoms and high fever, but with few parasites present in the blood and internal organs, and which were usually considered to be cases of pernicious fever with few parasites, this giving rise to the theory of the greatly increased virulence of the parasite under these conditions.

That sunstroke may complicate malarial infection and give it a pernicious character, especially in persons worn out by overwork and in alcoholics, has been asserted by many writers who have studied endemic malaria in hot countries, or in the hot season in temperate climates. Sternberg, but especially Kelsch and Kiener, recognized this complication; indeed, they demonstrated it by very accurate clinical and anatomico-pathological observations. Sullivan (quoted by Sternberg) writes that exposure of the head to the sun's rays, and a continuous high temperature day and night, such as is found in the tropics, especially when evaporation is interfered with by moisture in the air, favor the development of pernicious fevers.

Even in temperate climates, in both city and country, cases of insolation occur on hot, suffocating days, especially in debilitated patients unused to hard country labor, or those weakened by the immoderate use of alcohol. In our Campagna, we sometimes see cases of this trouble, but rarely. It is probably to insolation that are due those cases of disease which are attributed to some unknown infection, which we sometimes have occasion to observe in the hospitals of Rome during the hottest portions of July and August, and even in the first half of September. The disease attacks men working in the country at threshing or at preparing the ground for sowing, who work too arduously and too long and who are poorly nourished. The patients are carried to the hospital in coma or in delirium; the disease lasts a few days or a week at most, and the autopsy shows cerebral or pulmonary congestion, degeneration of the liver and kidneys, dilatation of the heart, etc. Now in addition to these cases of pure insolation, we have some of malaria complicated by insolation. This may occur even during an ordinary febrile attack, when the workingman taken with a chill lies down on the ground in the sun; and there he is likely to remain in sopor or in coma until he is transported to the hospital. In cases of coexisting malaria and insolation it is less difficult nowadays to decide which symptoms are due to the one disease and which to the other, because we can have recourse to an examination of the blood; by this means also we are able to decide which are the chief factors and which the accessory.

In conclusion we may add that in malarial climates insolation either produces the whole disease, which has a varied symptomatology and a varied course (acute, subacute, and chronic—Hirschfeld), or it is complicated with malarial fever. In the first event, the characteristics of malarial infection are entirely absent; in the second the infection may be very light, as is shown by the few parasites present and the slight visceral melanosis; or it may be grave, as happens when a patient suffering from pernicious malaria remains exposed to the sun's rays, or when during the disease caused by insolation infection with many estivoautumnal parasites occurs.

DIAGNOSIS.

In the diagnosis of malarial infection we have to consider the species of the infection and the special form of each species.

Mild Malarial Infection.—In simple tertian or quartan fever the diagnosis, except in very rare cases, is easy, if we give due consideration to the season of the year, the place of sojourn, and the occupa-

tion of the patient, as well as to the places where he may have been staying one, two, or three weeks before the appearance of the fever; and if, moreover, we carefully consider the distinctly intermittent and periodical fever which begins with more or less intense chill and ends in sweating, the type of the fever being variable (simple, double, and triple quartan; simple and double tertian). In these markedly intermittent fevers an examination of the blood almost always permits of a quick diagnosis not only of malaria, but also (as may be deduced from what has already been said in regard to the morphological and biological characteristics of the quartan and tertian parasites) of whether the infection is a tertian or a quartan, whether it be simple or complex, and approximately in what period of the fever or apyrexia the patient is. It will be well to bear two things in mind: first, that while the quartan parasite exhibits every phase of its existence in the peripheral blood, the same is not true of the tertian, whose fission phase is sometimes not to be seen at the beginning of the attack; second, that in the first attacks of a tertian fever the parasites may be very few in number, or, according to some authorities, even be entirely wanting in the peripheral blood; this scarcity or absence of parasites is also observable in the first attacks of an experimental tertian.

But although an examination of the blood may be omitted in the majority of cases of simple quartan and tertian, it is most useful and sometimes even necessary in the quotidian fevers of tertian and quartan origin, and in the rare subcontinuous fevers from the same source, especially in the summer and autumn, in order promptly and clearly to distinguish it from fevers of the same type belonging to the estivo-autumnal group. In these fevers the splenic tumor is scarcely appreciable in the first attack, but in the relapses the spleen usually projects below the border of the ribs, and is easily palpated.

In chronic infections belonging to the simple tertian or quartan groups, the earthy pallor of the patient in itself permits of a diagnosis; but when the intervals separating the attacks are long, when the appetite is keen, and the hygienic conditions surrounding the patient are good, then this earthy pallor may be wanting.

In infants and children, even those who have had the fever but a short while, anæmia is frequent, and so is anasarca, even without there being any nephritis.

Estivoautumnal Infection.—The same general data are useful in the diagnosis of this form of fever. As to the season, we must remember that it is only in the summer and in the autumn that the first invasion of fevers belonging to this group is manifested in a temperate climate. Relapses may occur at other seasons, especially in

the winter, when it is altogether exceptional to see cases of primary infection. As to the dwelling-place of the patient, it will be well to note that even in regions where every variety of the infection is manifested in the summer and in the autumn, there are certain localities where grave infections are more frequent, and others where the milder types prevail. We should be careful to observe whether the house is in the centre or at the outskirts of the settlement; if the latter, sleeping with the windows open may give occasion to infection.

The type of the estivoautumnal fevers is frequently less regular than that of the tertian and the quartan. The quotidian type is easily recognized, even when the initial chill is not so marked as in the quartan and in the quotidian of tertian origin; indeed, this short and not severe chill is in itself a symptom which serves to distinguish the first from spurious quotidians. The estivoautumnal tertian is not so easy to diagnose, as is proved by the fact that only lately has it been recognized in the various malarial regions, and some authorities even yet do not admit its existence, or at least do not recognize its tertian origin. The frequent absence of an initial chill, the long duration of the attack, the oscillations in the temperature curve, especially at the pseudocrisis and the crisis, the brief and obscure periods of apyrexia, are and always have been the chief difficulties in the recognition of this type. For the diagnosis of this tertian it is necessary that the temperature be recorded at least every four hours. If this were not done, and especially if it were taken only twice a day, the attending physician might well believe that he was dealing with a case of continuous fever with morning remissions or, if the pseudocrises were marked, with a quotidian fever. Moreover, we must bear in mind that the estivoautumnal fevers are in the beginning irregular and subcontinuous and that they only later clearly reveal their type.

In the presence of a pernicious attack the diagnosis can be made if, in addition to the general data already given, we learn that there have been prolonged estivoautumnal tertian or quotidian febrile attacks, and that the onset of the pernicious symptoms came abruptly after the febrile invasion. The rapid disappearance of these symptoms with the cessation of the fever confirms the diagnosis. Thus it sometimes happens that in the evening we leave the patient in a state of coma, with a high fever, and even with labored and stertorous breathing, and in the morning find him conscious, peaceful, and apyretic, although debilitated. The diagnosis of non-febrile pernicious fever, which sometimes has a protracted course and variable symptoms, is often difficult, and demands much experience and ability unless we have recourse to an examination of the blood.

Examination of the blood, when one has acquired the skill necessary, and when it is patiently and carefully performed, will usually permit of the diagnosis not only of malarial infection in general, but of the estivoautumnal type in particular. It is true that the morphological and biological diversity of the parasites causes some difficulty in the diagnosis. In the quartan and tertian infections the parasite is easily recognized by the usually abundant amount of pigment present; the same may be said for the conspicuous crescent forms and the even more striking flagellated forms; but the small non-pigmented, motile, or annular amœbæ which are found in such abundance in these fevers require greater skill and experience for their recognition and their differentiation from the accidental vacuoles which are formed in normal red corpuscles. Even when the little amœbæ are pigmented, the pigment is so scanty and so powdery as to require the closest attention for its recognition; it is more easily recognized when in the form of blocks of pigment at the centre. The presence of necrotic parasite-infected red corpuscles, known as brassy bodies, is of much assistance to the diagnosis, but it is essential to know them well and not to confound them with the mulberry-shaped red corpuscles so frequently seen in improperly treated blood preparations. The life cycle of the estivoautumnal parasite has been described, the phases of its existence have been seen in the peripheral blood, and the relation of the same to the various stages of the fever and of the apyrexia has been noted; the reader has only to consult the description in a previous section to receive assistance in the diagnosis. One point, however, we desire to emphasize, namely, that *at certain moments, and especially at the beginning of the febrile attack, an examination of the blood frequently gives a negative result.* But even when parasites are not found in the peripheral blood, the diagnosis may be made by the presence of leucocytes and of endothelial cells containing pigment, parasites, and parasite-infected red corpuscles. Moreover, while in recent malignant tertian infections the pigmented leucocytes are chiefly seen during the attack and in greatest number about the time of the precritical elevation of temperature, when the infection has already lasted some time pigmented phagocytes are found at every examination, even four, five, and eight days after the parasites have disappeared, especially in grave infections.

We spoke in detail of the contents of the blood in pernicious infections in the section treating of that form of the disease; there we said that in pernicious fevers the number of parasites is usually great, that the forms with blocks of centrally situated pigment are also relatively abundant, and that we sometimes see sporulating bodies.

But we must also remember that the pernicious condition can persist even with diminution and disappearance of the parasites in the blood of the periphery.

That there are cases of estivoautumnal fever in which it is impossible to find any parasites has been emphatically stated, and we have ourselves mentioned that they may be present in small number. Here we would insist upon the fact that before positively asserting that there are cases of grave malarial infection without parasites in the peripheral blood, it would be well to make further accurate microscopical and clinical researches in cases in which there arises the necessity for a differential diagnosis, and to realize that in order to warrant a statement that results are negative, the examination of the blood must have been accurate and painstaking, made in several specimens prepared at various periods of the fever and of the apyretic condition, and not made in a hurry during one period only of the attack, especially the invasion. From our own experience and that of our colleagues, we are inclined to hold that when the results of the blood examination are truly negative, the cases are not of pernicious malaria, but of some other infection or of some obscure form of autointoxication.

That an estivoautumnal infection has already lasted several days may be ascertained by the presence of crescent forms, and of those forms which immediately precede or follow them, and which are easily recognized. They are found alone or with amœboid bodies: alone in the apyretic periods intervening between the first invasion fever and a relapse and between the relapses, and with amœboid forms during the febrile attacks.

Puncture of the spleen for the diagnosis of malaria, although when skilfully done quite free from danger, and furnishing an easy method of diagnosis, should be performed only in exceptional cases, when a differential diagnosis is absolutely necessary. Whenever we have extracted blood from the spleen, it has not been to receive assistance in the diagnosis, the circulating blood having been all sufficient for that purpose, but purely in the interest of science.

Malarial Cachexia.—The diagnosis of malarial cachexia offers no difficulty, if we give careful consideration to the locality where the patient has been staying, the length of time that he has been suffering from the fever, the enlargement of the spleen and the liver, the earthy complexion, and the poor condition of the blood with its characteristics of secondary anæmia. The parasites are present in the blood during febrile attacks, crescent forms being found in addition to the amœboid.

Differential Diagnosis.

The differential diagnosis of malarial infections, especially of some of the clinical varieties, has been one of the most discussed points in pathology, and is the field upon which the ingenuity of clinicians has been most displayed. And rightly so, because the great variety of malarial fevers as to the clinical type and its method of behavior, the multiform symptoms of pernicious fever, and the existence of hidden or larval forms, made it necessary that the practitioner should possess data sufficient to enable him to differentiate the various forms of malarial fever from those of other diseases. Since the examination of the blood has been proved to be of such invaluable assistance in diagnosis, this latter has become a much easier matter. Thus we may suppose that a sufferer from heart disease has an intermittent quotidian or tertian fever and an enlargement of the spleen leading to a suspicion of malaria, and yet that the results of examination of the blood are truly negative. We can then exclude malaria entirely, and on giving increased attention to the heart may discover that the case is one of recurrent endocarditis with an intermittent fever.

A quotidian intermittent fever occurs in a patient suffering from chronic catarrh of the bladder. The question arises, Is this a fever secondary to the cystitis or to a complication of the cystitis, or is it an intermittent malarial fever? An examination of the blood shows a double generation of tertian parasites, and small doses of quinine cause cessation of the fever. These examples might be indefinitely multiplied; indeed, the cases which we have already reported at some length contain many things that show the importance of examining the blood. The finding of one parasite only suffices for the diagnosis of malaria.

It will be useful to pass in review the diseases which can be and which have been in the past confounded with malaria. In the first place come those which are accompanied by intermittent fever—these are suppurative conditions, some forms of puerperal fever, ulcerative endocarditis with a subacute course, biliary calculi, pneumonia, tuberculosis, influenza, typhoid fever, hysteria, and many other affections.

In *internal suppurations*, as abscesses of the liver, purulent paranephritis, etc., the fever is often intermittent, and even periodical of quotidian type, with chills and sweating. The history of previous diseases, the pains, the physical signs, and the inefficacy of quinine will all serve as guides to the diagnosis.

In malarial climates, *ulcerative endocarditis* with its protracted

course, with intermittent quotidian or tertian fever, chills, and sweating, not infrequently has every appearance of malarial infection. The physical signs of the heart, the negative effect of quinine, and sometimes the occurrence of cerebral embolism enable us to recognize the true cause of the intermittent fever.

In *cholelithiasis*, after one or several attacks of hepatic colic, even when the jaundice is diminished or disappears, there may appear a series of intermittent fevers lasting for days, weeks, and even months; the attacks occur at different hours of the day, are short, and sometimes two or three of them are seen within the twenty-four hours. The preceding colic, the pains in the hepatic region, and the jaundice indicate that the intermittent fever is of hepatic origin; moreover, it ceases soon after the passage of the calculus, although it has obstinately refused to yield to quinine.

In *pneumonia* the fever may be remittent; the pain, the nature of the sputum, and the signs of hepatization of the lung permit of a diagnosis of pneumonia, while an examination of the blood excludes even a complicating malaria.

The course of *tuberculosis* is often accompanied by intermittent fever, which is especially liable to be interpreted as of malarial origin if the symptoms of the original disease happen to be but slightly or not at all manifest. Perplexity will be of but short duration, however, for the gradual emaciation, the absence of earthy coloring and of splenic tumor, and the results of a careful physical examination of the chest, with other symptoms unnecessary to enumerate, will soon solve the question.

In the recent epidemics of *influenza* occurring in this neighborhood, more particularly in that form of it which was accompanied by irregular intermittent fever, and especially when the type was regular (quotidian for instance), malaria was often suspected, and quinine was therefore given, the inefficacy of which, however, in cases of influenza was soon clearly demonstrated. The knowledge that there is an epidemic of the kind, the season of the year, and the presence of catarrhal symptoms will all aid in the diagnosis, but it must not be forgotten that influenza may rouse a latent malaria or favor a relapse, whence the necessity in certain cases of clearly distinguishing intermittent malarial fever from postinfluenzal fever, which, as we know, may last for several weeks.

Hysterical fevers, the diagnosis of which calls for much judgment and clear perceptions, may have an intermittent course. The stigmata and the other symptoms of hysteria, the exceptionally high temperature (even up to 44° C. = 111.2° F.) without a corresponding rapidity of pulse and respiration, and absence of the febrile

characteristics of the urine, will enable us even in the most unusual cases to make a proper diagnosis.

A mere mention will suffice of the other diseases whose temperature curve might make us think of malaria; these are urethral fever and renal fever; the latter, which is analogous to hepatic fever, is manifested in cases of renal calculus, and it may be intermittent or continuous for several days when the renal colic is persistent.

In *typhoid fever*, at the time of defervescence, when the fever has become intermittent, or in those cases in which the temperature follows an erratic course, or when the curve is frankly intermittent, there are (and even more there were in the past) good reasons for difficulties in the way of a diagnosis, and also for errors. At the present time an examination of the blood on the one hand and serum diagnosis on the other have smoothed the path to a correct interpretation of that which formerly was subject to so much doubt.

There are also some infections which, in spite of having a continuous fever, possess some resemblance to grave malarial infection with irregular or subcontinuous fever. Two especially are liable to be confounded with malaria, namely, typhoid fever and febrile icterus. It is true that at the present time, although more doubtingly than of old, we think primarily of malaria in some cases of *typhoid fever*, and more often of typhoid fever in some cases of subcontinuous malarial infection. The first occurs in malarial regions, and in the hot season when the course of the typhoid fever is irregular, diarrhœa is absent, the spleen is much enlarged, and there is no characteristic eruption. But the duration of the disease in spite of the administration of quinine, to say nothing of the results of an examination of the blood, should convince us that the affection is not malarial. But in order to judge correctly, physicians should rid themselves of the notion of the prolonged and obstinate resistance of typhoid-like subcontinuous malarial fevers to quinine. When quinine is properly administered the fever is not prolonged more than four, five, or six days (and be it noted that this limit of resistance is rarely attained), leaving aside the possibility of a spontaneous fall of temperature with recovery, which is rare in this form of the disease.

The second case, that of mistaking a malarial fever for typhoid fever, occurs in primary malarial infections when the fever is continuous from the beginning. On account of the error in diagnosis, the fever may run on for days and weeks, with remissions and short intermissions; and finally, if death do not occur, the temperature curve may assume a tertian or quotidian type. We recall one case of a young hunter suffering from a subcontinuous fever, which had come on as such primarily and had been regarded as typhoid; the tempera-

ture curve became of an intermittent quotidian type and was interpreted as the last stage of the typhoid infection, but an examination of the blood showed the parasites (amœboid and crescent forms), quinine was given, and the fever fell. These errors in diagnosis occur especially when typhoid fever is raging during the malarial season.

The differential diagnosis between typhoid fever and typhoidal subcontinuous malarial fever was clearly formulated some time ago by Baccelli; we have given it in our description of the subcontinuous fevers. At the present time, the examination of the blood and Widal's test are indispensable for settling the question of diagnosis.

Another disease which, during the summer and autumn in malarial countries, has often been diagnosed as subcontinuous malaria or subcontinuous icteric fever is *febrile icterus*, now also called *Weil's disease*, although the disorder was well known previous to the descriptions given by this clinician. The mistake is the more easily made because febrile icterus sometimes occurs in slight epidemics in the hot season, at the time when grave malarial disorders are in the ascendant; this sometimes happens in Rome, the disease by preference attacking river fishermen who carry on their business in malarial regions. Increase in size of the spleen, and sometimes also of the liver, seems further to confirm the erroneous diagnosis; while albuminuria, jaundice, and muscular pains may also be found in malaria. But the continuous nature of the fever with regular evening exacerbations, the often severe muscular pains, especially in the calves of the legs, jaundice, and decolorization of the fæces (in malaria, deep staining of the dejecta accompanies jaundice), and the daily occupation of the patient enable us to distinguish febrile icterus from malaria. Examination of the blood is always useful in this disease, but it is of the very greatest importance when the patient comes from the country and has been working in the fields. We had the opportunity once of seeing a case of this kind, the patient being a young man who for several consecutive days had been superintending certain work in the Roman Campagna, in a locality where several workmen had been attacked with malaria. This was in the month of August, and he also was taken with the disease, after having overworked himself, partaken of poor nourishment, and drunk water for several days from a well in which there was a bad odor. Malaria was suspected at first, but an examination of the blood completely excluded it. The disease, accompanied by muscular pains, jaundice, tumor of the spleen and liver, light-colored fæces, urine of an intense yellow color with little albumin, frequent pulse, and cutaneous and conjunctival hemorrhages, lasted for ten days, was grave and some days extremely grave in its nature, and was accompanied with nervous phe-

nomena and acute dilatation of the heart. After eight days of apyrexia there was a relapse with return of the fever, the icterus, the nervous symptoms, and albuminuria; the course of this relapse was long, but it finally ended in recovery.

The differential diagnosis between the pernicious attacks and the diseases whose symptoms resemble them is not always easy, and calls for skill, experience, and promptitude, the latter especially because in these cases *periculum in mora*. It is true that, as some physicians say, we can secure ourselves by the administration of quinine; but although this advice may be followed in some cases, it is certainly better when the means at our command permit it to make a prompt diagnosis, have a sure basis for the prognosis, and be able to decide whether the quinine be really indicated.

Cerebral apoplexy, insolation, meningitis, tetanus, acute bulbar paralysis, acute delirium, cholera, dysentery, uræmia, scarlatina, grave hemorrhagic infections, and still other diseases must be taken into account by the physician at the moment of danger.

Cerebral apoplexy may be suspected in cases of pernicious malarial fever with profound coma, relaxation of the muscles, hard and slow pulse, abolition of the reflexes, labored and stertorous breathing, etc. The season of the year, the place where the patient has been staying, the age of the subject, the high fever, and the enlarged spleen will lead to a correct diagnosis. Less easy of recognition are the rare forms of pernicious fever with stupor and hemiplegia and without fever; but as this is more likely to occur in the relapses in an anæmic patient, the small and frequent pulse, the anæmia with earthy pallor, the free respiration, and the enlarged spleen will suffice to a proper diagnosis.

Sunstroke may simulate an attack of pernicious fever, in malarial climates, and in the summer, when it attacks men working in the fields. But the conditions under which the disease occurs, the sometimes fulminating manner in which cerebral symptoms appear, and the absence of preceding febrile attacks and of enlargement of the spleen will preserve us from error, even if an examination of the blood be not made. But we already know that insolation may complicate malarial infection, and give it the appearance of pernicious fever, when the grave symptoms are really due to the sunstroke alone. These are some of the diagnostic difficulties which call for good judgment and experience.

Meningitis, especially acute cerebrospinal meningitis, may be suspected in pernicious fevers with meningeal symptoms, especially if the patients are young and robust. Here also the season, the absence of epidemics of cerebrospinal meningitis, the precedent attacks,

the abrupt onset of the symptoms, etc., indicate pernicious fever. We have seen that malarial infection may coexist with tuberculous meningitis, and in the cases described, while an examination of the blood inclined the physician to diagnose the former, the autopsy showed combined tuberculous meningitis and malarial infection. In these cases the greatest difficulties are experienced when we cannot ascertain the occurrences which took place before the patient fell into the present condition; but when we know the prodromes, the preceding stages of the disease, its duration, and the successive symptoms, when we carefully examine the internal organs, and when we find parasites in the blood, we can make a diagnosis between the two diseases, especially when the parasites disappear under treatment by quinine, leaving the meningitis to pursue its course.

Tetanic pernicious fever recalls the acute form of *tetanus* with high temperature; but the absence of external lesions, the stupor, the brief duration of the disease, and its abrupt onset will help us to diagnose pernicious fever.

In bulbar pernicious fever, when we do not know the previous history we may be led to believe that the affection is acute *bulbar paralysis*. The high fever, or rather the occurrence of the bulbar symptoms only after the febrile invasion, the absence of hemiplegia alternans, the rapid disappearance of the symptoms and the marked improvement after the attack of fever, in addition to the age of the patient, will admit of a diagnosis of febris perniciosa even without examination of the blood.

The differential diagnosis between genuine *acute delirium* and delirious pernicious fever rests upon data already several times described. The difficulty is increased if the acute delirium appears when the parasites have disappeared from the blood and the temperature is high. An exact estimation of all the events which have preceded the manifestation of the delirium and those which accompany it, especially the earthy complexion, the splenic tumor, and the final presence of pigmented leucocytes in the blood will throw light upon the path.

During epidemics of *cholera* in the summer and the autumn in malarial countries, the practitioner is often perplexed between the diagnosis of cholera and that of choleraic pernicious fever in the case of patients who are cold and cyanosed, who have copious diarrhœa, cramps in the calves, and the Hippocratic facies. An examination of the blood is of the utmost value in these cases, but it should not be forgotten that the same patient may have both cholera and malaria.

What we have said in regard to choleraic pernicious fever is to be

repeated for *dysentery*. The diagnosis of this form of pernicious fever is made from the rapid appearance of the dysentery, the high fever, and the cessation of the sanguinolent diarrhœa with the ending of the fever.

The differential diagnosis between cerebral pernicious fever and *uræmia* is seldom called for. Of course it will be readily understood that the uræmia in œdematous nephritis with scanty urine rich in albumin, casts, etc., is not the variety which can concern us, but rather the uræmia which suddenly occurs in cases of chronic nephritis without œdema and with apparent well-being in robust individuals. The low specific gravity of the urine, the small amount of albumin, the nature of the pulse, the signs of hypertrophy of the heart, the absence of splenic enlargement, and often of the fever are all symptoms pointing to uræmia. It will be well to remember that cases have been noted in which there was a subacute or chronic nephritis complicated by malarial infection, which was not recognized because all the symptoms, including the fever, were attributed to the first-mentioned disease.

We have described hemorrhagic pernicious fever, in which the gravest symptom consisted of hemorrhages in the skin and the mucous membranes, and which might continue for two or three days in malignant tertian pernicious fever. Now, in these cases we may diagnose *purpura hæmorrhagica* in its hyperacute form with high fever. We once found ourselves in this diagnostic difficulty in a case of hemorrhagic malarial infection in a woman, early in the winter season; but the doubt was soon dispelled by an examination of the blood. It might also happen during the season of grave malarial infection that we should mistake purpura hæmorrhagica, or some other hemorrhagic disease, for malaria. We once saw a case in a young girl of *acute leukæmia* accompanied by cutaneous and retinal hemorrhages of the stomach, the gums, the genitalia, etc., in which hemorrhagic malarial infection had been diagnosed because there was also an irregular fever which went above 40° C. (104° F.) and because there was an enlarged spleen. An examination of the blood did not show any malarial parasites, but there was an enormous increase in leucocytes. Unfortunately we were not able to make the careful examination of the blood which was called for in this case, nor the autopsy.

In the rare cases of erythema diffused over the whole body accompanying grave malarial infections, we might for a little while suspect *scarlatina;* but the further course of the disease, the frequent relapses of erythema, the intermittent fever, etc., enable us finally to exclude it.

It would be superfluous to go farther into the differential diagnosis, because what we have already stated seems to us to be sufficient to serve as a guide in other doubtful cases, and also because in these days the new light shed by the examination of the blood renders the diagnosis much less difficult than it used to be.

In malarial cachexia the diagnosis is to be made between it and the diseases whose chief symptoms are enlargement of the spleen and anæmia, especially if these are accompanied by intermittent fever. These diseases are splenic leukæmia and splenic anæmia.

In *splenic* or *splenicomedullary leukæmia* there is often a quotidian intermittent fever; and because of this, and the progressive increase in size of the spleen, we are inclined in malarial countries to judge the case to be one of chronic malarial infection. An examination of the blood showing the increase in the number of leucocytes will settle the question; but it is not to be forgotten that sometimes, although rarely, leukæmia may develop in a malarial patient, and that in this event an examination of the blood will reveal the coexistence of the two diseases.

In *splenic anæmia*, the differential diagnosis is sometimes less easy. We will not enter into the question as to whether splenic anæmia, which means an essentially progressive anæmia, accompanied by a splenic tumor in which the cause of the disease seems to reside, should or should not be considered merely as a splenic pseudoleukæmia, but will simply say that on account of its clinical, anatomico-pathological, and therapeutic characteristics we hold that splenic anæmia is a disease by itself, which cannot be classed with the pseudoleukæmias, of which the glandular form only is well known. In splenic anæmia, the enlargement of the spleen, the progressive anæmia, and sometimes the febrile attacks in which the anæmia and the splenic tumor increase, while both may diminish in the periods of apyrexia, are symptoms which strongly remind us of chronic malaria. The same mistake may occur in the *splenomegaly with cirrhosis of the liver of Banti* not only in the anæmic period, but also in the ascitic, because ascites may be considered to be the result of hepatic atrophy, such as not infrequently occurs in advanced malarial cachexia. The careful study of the general data which we have already several times enumerated, and the examination of the blood, in which parasites are found when the cachectics have intermittent fever, will make the diagnosis easy. But we must bear in mind that in some non-febrile cachectic conditions of malaria, parasites are no longer to be found in the blood.

PROGNOSIS.

In giving a prognosis in malaria, we have to consider the active infection, the sequelæ, and the complications.

The prognosis during the infection relates to the nature of the infection, including the place and the season in which it was contracted, as well as the individual conditions.

In mild fevers—*tertian* or *quartan*—the prognosis is good; as we have shown, pernicious infection does not occur in these types of fever. There may be attacks of an apparently grave nature, with severe headache, vomiting, labored breathing, great thirst, etc., but since the discovery of the parasite no one has described a pernicious fever of simple tertian or of quartan origin. The autopsies in these cases have always been made upon subjects who have died of some intercurrent disease; indeed, in this connection, we have already described a case of Barker's, in which it was shown that a simple tertian infection which for several weeks was unrecognized in a patient suffering from nephritis with streptococcic septicæmia at the last, gave rise to not one of the known symptoms of pernicious fever, although a post-mortem examination of the blood and of the organs revealed an enormous number of simple tertian parasites.

In infants and children, in whom treatment is neglected from carelessness or poverty or from a prejudice against quinine, we have secondary anæmias and also anasarca, but these symptoms rapidly disappear when the infection is exhausted naturally or by treatment.

In the prognosis of these fevers, we must also take into account the fact that relapses may occur for a long time, and that if the patient lives under bad hygienic conditions cachexia may follow, and complications or morbid sequelæ may occur, among them nephritis and amyloid degeneration.

We have stated that recovery from these fevers is sometimes spontaneous, as all the recoveries from malaria used to be before the introduction of the specific remedy into therapeutics. As to the changes in the parasitic contents or the final modifications in the morphological elements of the peripheral blood, we know of nothing that could be of any assistance to the prognosis of a spontaneous cure. The only well-ascertained change revealed by an examination of blood from the finger is the gradual diminution in the number of the parasites. Whether during the progress of these fevers towards a spontaneous cure the phagocytes increase, whether they contain young parasitic forms, and whether the free degenerative bodies increase in

the blood, from the observations so far made we are unable to state positively enough to be of any use to the prognosis.

As to the behavior of the fever in cases of spontaneous recovery, we may state that there is usually a gradual diminution in the severity of the attacks; but the occurrence of attacks with a higher temperature should not necessarily give rise to a belief in an aggravation of the disease, because it sometimes happens that some one of these attacks is followed by a cure. Moreover, we must not think that after spontaneous recovery immunity from relapses has been acquired. On the contrary, we not infrequently see cases of spontaneous cessation of tertian fevers many times in the same person followed by relapses after twelve, fifteen, or twenty days, or even later.

In quartan and tertian fevers, especially in the former, an examination of the blood will enable us to predict with certainty whether an attack is imminent, and usually from the number of the parasites whether it will be light or severe, in reference, of course, to the height of the fever and the non-pernicious symptoms accompanying it.

In the *estivoautumnal fevers* prognosis should be reserved, if for no other reason because of the possibility of a pernicious infection. Even in these fevers we sometimes have a spontaneous cure. As to the modifications in the blood discernible on a microscopical examination, we can only repeat what was said with reference to the prognosis in mild fevers, adding merely that in many cases the appearance of or an increase in the number of crescent forms seems to coincide with the spontaneous cure. We must not, however, suppose that their presence always indicates the near approach of recovery because if the formation of the crescents is followed by an increase in the bodies of the febrigenous cycle, the fever will continue, and may even become pernicious. Many cases of pernicious fever, even lethal ones, have been observed by us in which the crescent forms were numerous in the blood and in the spleen, and exceedingly numerous in the bone marrow where the various stages of their development could best be followed. Neither should we believe that spontaneous cure is always accompanied by the appearance of crescent forms. We remember, indeed, to have seen the spontaneous cure of a group of winter relapses, especially delayed ones, without having succeeded in finding crescents in the peripheral blood or even in the spleen.

Can we predict the imminence of a pernicious attack? Of course, whenever such a prognosis is made preventive measures are at once resorted to, but unfortunately we do not always succeed in making the prognosis. A clinical examination in the preceding attack will sometimes show that among the ordinary symptoms are the prodromes of some that will become pernicious, prodromes which, as Torti says,

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are the "*exiguus mus qui ferale dabit augurium*"; prodromes which persist, and even become aggravated, during the short and unsatisfactory period of apyrexia which holds and matures the germs of the imminent pernicious attack. Drowsiness, replying with effort to questions, loss of memory, apathy, dulness, etc., are signs which should lead us to suspect cerebral pernicious fever. An examination of the blood during the period of apyrexia will sometimes enable us to foretell the occurrence of an attack, even when we are unable to prevent it. The presence of a large number of parasites (in one case about half of the red corpuscles were affected), many of which are mature, with central blocks of pigment, and near sporulation or even actually in sporulation, is one of the signs which, in spite of large doses of quinine promptly administered, are usually followed by pernicious attacks, even fatal ones.

What prognosis shall the physician make when the pernicious fever is already developed? If the particular attack under observation has been preceded by another, the prognosis will be graver, although there have been cases of recovery from second and even from third pernicious attacks. Whether the pernicious attacks have appeared in the original infection or in a relapse seems to be of no prognostic importance, excepting in cases in which the patient is profoundly anæmic and the number of parasites is exceptionally large. The prognosis should, moreover, be based on a careful examination of all the symptoms, especially those which relate to disturbances of the nervous or circulatory systems. Profound coma with labored, interrupted, or stertorous breathing, dilatation of the heart, signs of collapse, especially algidity and cyanosis, with cold and clammy sweat, etc., all indicate to the physician how guarded must be his prognosis. If then an examination of the blood shows numerous parasites, many of them containing central blocks of pigment, we may with certainty predict further aggravation and prolongation of the attack. But experience teaches that in some cases neither the diminution in the number of the parasites nor their disappearance from the blood of the periphery will warrant a favorable prognosis. The reason for this we already know. Either the parasites have taken refuge in great numbers in the small blood-vessels of some important organ, as the brain, or the organism (as we are obliged to believe in the present status of our knowledge) succumbs to a profound intoxication.

The prognosis will vary according to the form of the pernicious attack. No pernicious fever is absolutely and necessarily fatal. A comatose perniciosa with small and frequent pulse and stertorous breathing, or a choleraic perniciosa with algidity and cyanosis, may sometimes terminate in recovery. According to our experience, as

well as that of others, the pernicious fevers arranged in the order of their relative gravity would be cerebral, choleraic, and algid. As regards these forms of the disease we recall what was said above as to the insidiousness of their course, and how the perfect tranquillity of the patient would give us no reason to suppose that a fatal result was imminent. A filiform pulse, cyanosis, algidity of the whole body, and a cold breath are symptoms calculated to deprive the physician of all hope.

Experience will teach us whether a symptom is likely to disappear wholly or in part at the end of the attack. Thus in the group of cerebral pernicious fevers, it is frequently observed that certain nervous symptoms, already enumerated in the section on morbid sequelæ, persist for a variable time after the attack. On the other hand, in intestinal and algid pernicious fevers the symptoms usually disappear with the cessation of the attack.

In the prognosis of estivoautumnal infections we have to remember the frequency of winter relapses, which often continue to reappear at short intervals until the spring. We have already said that it was a rare occurrence for pernicious fevers to make their appearance in winter relapses, and that there often was a gradual attenuation of the attacks until complete recovery was attained. As to the examination of the blood, we have also already stated that when crescent bodies were found they usually diminished and even disappeared entirely at the approach of each new attack, to again return or increase at the beginning of the intervals of apyrexia. It is important to know this fact, in order that we may not think that the infection is spent simply because we notice the diminution or the disappearance of the crescent bodies.

The prognosis varies with the malarial region according to the kind of infection which predominates in such a locality. It is evident that an infection contracted in a region of mild malaria should have a different prognosis from that taken in a place where the malaria is grave in its nature. But since there are districts, not always of great extent, where there are circumscribed endemics of malaria, where in the summer and autumn every variety of malarial infection can be contracted, the prognosis can be based only upon a knowledge of the nature of the infection in each individual case.

By this distinction of localities into regions of mild or of grave malaria we can understand the variety in the mortality of the various malarial districts. This explains why, in the plains of Lombardy, even in the summer and autumn, there is not one death from malaria, while during those seasons we have a greater or less number of deaths in the Tuscan coast region, the Roman Campagna, the Pontine Marshes,

etc. In the hospitals of Rome, to which the fever sufferers flee from the Campagna, autopsies upon those who have died of pernicious fever are made every year, while not one is made in Pavia and Milan (Golgi).

The *mortality* of some malarial regions is truly appalling. Laveran states that in the Madagascar expedition of 1895 the French troops lost six thousand men by disease in a few months (about a quarter of the active force), of whom seventy-two per cent. died of malaria. But while recalling these and other instances of slaughter from malaria in armies fighting in malarial regions, we must take into consideration the fact that in time of war there are many factors which favor the infection, render it more grave, and interfere with regular treatment.

As to the prognosis in relation to the season of the year in which the disease occurs, it is clear that in temperate climates where every variety of the infection may be contracted, the prognosis must vary according to whether the disease is initiated in the spring, in the summer, or in the autumn.

As in all infective diseases, so in malaria, the prognosis varies with individual conditions, such as race, age, constitution, social conditions, and previous or existing diseases.

It has been asserted by many authorities that negroes do not easily contract malaria, and that when they do the course of the infection is short and not severe. In proof of this we have the statements of William. In 1841 three English vessels, the *Albert*, the *Wilberforce*, and the *Soudan*, went up the Niger; their crews consisted of 145 whites and 158 negroes. One month after their entrance in the Niger, 130 of the 145 whites were affected with the fever, of whom 40 died, while of the 158 negroes only 11 had slight attacks of fever, and not one died. Of these blacks, 133 came from the coast of Africa, and none of these was sick; the other 25 came from the West Indies, or from the United States, and had spent some time in England, and to this group the 11 sick men belonged. Although Sternberg and others criticise William's report as not clearly demonstrating that malaria alone was the cause of the sickness and death of so many of the white men, yet other statistics are not lacking from various parts of America and Africa to prove the greater resistance of the black race. Recently Koch has confirmed not only the fact of the comparative immunity of the negroes on the western coast of Africa, but also of the mild course of estivoautumnal fevers (tropical fevers of Koch) in them and the frequency of a spontaneous cure. The other dark-skinned races do not seem to share in this resistance to malaria.

In the aged estivoautumnal fever, if not promptly recognized and

treated, finds a ground suitable for the production of pernicious attacks, especially the cerebral variety. In infants and children, cerebral pernicious fevers, particularly the convulsive, are not rarely seen; and non-treatment of the disease results in rapid anæmia accompanied by anasarca.

It is natural that persons with enfeebled constitutions, whose circulatory organs are less active than normal, should more easily become subject to malaria than the healthy and the strong. For these and other reasons, the inhabitants of certain regions oppose greater resistance to malaria than do others, as may be seen in the Roman Campagna, where workmen from all parts of Italy are employed in field work.

The social condition of malarial patients has great influence on the prognosis. It being evident that when the infection is promptly recognized and treated not only does recovery ensue, but pernicious attacks may nearly always be averted, we can easily understand that a quick diagnosis and the intelligent and prompt application of remedial measures are much more easily effected in the case of persons in easy circumstances who can take care of themselves and follow treatment than in poor laboring men who, neglected by others and by themselves, continue with their work as long as they have any strength to do so. This explains those cases in which laborers are attacked by pernicious fever when going to their place of work or when returning from it, or while engaged in labor; and also why so many laboring men are brought by policemen or other persons to the hospitals, who have been found in the streets suffering from an attack of pernicious fever. It also explains the insolation which complicates a pernicious attack, when the patients during the chill lie down in the sun and remain there for hours in a state of sopor or coma. In soldiers the prognosis will vary according to whether it be a time of war or of peace. In times of peace the soldier is under surveillance, presents himself to the physician at the onset of the first febrile attack, and is promptly treated, whence the mortality from malaria is almost *nil*. In war times, on the other hand, the conditions under which the soldiers exist, and the difficulties in the way of prompt diagnosis and treatment, increase the sick rate and the mortality of malaria, and explain the actual slaughter sometimes caused by malaria in certain armies, as for instance the French army in the last war in Madagascar.

When malarial infection invades an organism convalescing from some previous disease or suffering from some affection, its resistance will, of course, be lessened; the multiplication of the parasites will be more active, grave attacks will be more liable to occur, the anæmia

will be of a more severe type, and the reconstitution of the blood will be longer delayed.

The *prognosis of the sequelæ* may be readily deduced from the descriptions of them which we have already given, for which reason we will here limit ourselves to a few brief observations.

Most of the nervous symptoms are recovered from, whether they be those which persist in an attenuated form after cerebral or cerebrospinal pernicious attacks, or whether they develop after the infection. Their duration varies greatly within limits of several months, but it is usually short. Yet after grave cerebral pernicious attacks with a prolonged course, and slow recovery from the secondary anæmia and the nervous symptoms there is apt to be weakness of the nervous system and even of the intellectual faculties.

The prognosis in the cases of splenic and hepatic tumors, even when they are very large, is that they are likely to diminish gradually in size; and if they persist after the infection has disappeared, frequently they cause no disturbance in sanguinification nor in the hepatic functions. Everywhere we may see workingmen who have been cured of a long-existing malaria, and in whom the enlargement of the spleen and even of the liver persists, whose appearance is healthy, whose muscles are large and strong, and who work with energy. As to enlargement of the spleen, we must bear in mind the possible sequelæ, among which are ectopia and rupture.

The prognosis of postmalarial anæmia depends upon the form which it assumes. Secondary anæmias are usually cured in the end, but we must remember that they sometimes last for a long time, that months after the infection is spent the number of red corpuscles in some persons is much below the normal. In those rare cases in which a secondary anæmia is followed by pernicious anæmia the prognosis is always grave. The mere presence of nucleated red corpuscles does not suffice to a diagnosis of pernicious anæmia; gigantoblasts may be found, and even prevail over the normoblasts, and yet the anæmia may undergo rapid improvement with final recovery.

In uncomplicated malarial cachexia the prognosis is never necessarily fatal. If the patient is removed from the malarial region and receives appropriate treatment, with rest and good nourishment, there is always a gradual improvement and final recovery; of the cachexia no vestiges remain except enlargement of the spleen and liver, and this is greatly diminished. In places where there are circumscribed endemics of malaria, natural or induced amelioration in the local conditions may be effected, and the disappearance of the malaria then follows; when this takes place, after the interval of a year or

two the pale, weak, melancholy, and cachectic inhabitants have usually become healthy men without a trace of the former infection. Such a transformation as this occurred in a suburb of Senigallia, to which we have already alluded, where a malarial endemic which was most grave in 1896 and severe in 1897, by natural amelioration did not reappear in the spring and summer of 1898. The infants and children who in the previous years had been seen in their mothers' arms or wandering in the streets, in a profoundly anæmic condition, suffering from fever and even anasarca, last year were found to be rosy, lively, and robust. We have already told of the traveller to the Congo who returned to Europe in a profoundly cachectic condition, and who in two years became so robust as to be scarcely recognizable by those who had seen him only when he had cachexia.

In the prognosis of malarial cachexia we must, however, take into account the fact that the organism of the patient is favorable ground for the development of other infective diseases.

When in a cachectic there are symptoms of amyloid degeneration (abundant albuminuria, obstinate diarrhœa, uræmic symptoms, etc.), the prognosis is altogether bad.

The prognosis of *complications* does not require to be dwelt upon at length, because it can be easily inferred from the descriptions already given. In pneumonia of patients already weak and anæmic from malaria, the uncommon gravity of the symptoms, the facility with which complications with the diplococcus arise, the delay in resolution, and the liability of induration, etc., are all to be taken into account in the prognosis. The same circumspect attitude should be preserved in regard to the prognosis of other infective diseases which complicate active malaria, or make their appearance in cachectic patients. If malarial infection develops in a tuberculous or scrofulous subject, no faith should be placed in the theory of antagonism, because experience teaches us that tuberculosis in an organism which has become infected with malaria continues on its fatal path, and may even be hastened and become miliary. The complication of insolation with malaria makes the prognosis worse; indeed, in the cases which we have seen, although the infection was not of a grave nature, as shown by an examination of the blood, the ending was always fatal.

Prophylaxis.

Our recently acquired knowledge concerning the life of the malarial parasite outside of the human body will have as a result that, in consequence of the recognition of the cause, we shall be guided into the direct way of preserving man from infection. Up to this time we

have known only empirically the conditions favorable to malarial endemics, and we endeavored empirically also to remove these conditions by means of hygienic measures. The opinions held as to the seat of the parasite in the outside world and its manner of entering the human organism were various. According to some, the parasite lived in the earth, whence it rose into the atmosphere and entered the body of man by way of the respiratory passages. Others believed that its conditions of existence were found only in water, and that this was the sole vehicle of infection. But now it has been demonstrated that the parasite, when outside of man, lives in the bodies of certain diptera which suck human blood, and which inoculate man with the saliva during the puncture. All that we know of the life of these insects, and also epidemiological experience teach us that the parasite passes from the body of the mosquito into that of man only directly by means of inoculation, and make very improbable the hypothesis that the passage of the parasite from the mosquito to man may take place indirectly through the air or water, the latter being the medium through which filaria pass from insects to man. Of all this, as also of the life cycle of the parasite in man and the mosquito, we have already spoken in the sections on Parasitology and Etiology. But as regards the prevention of malaria, the most important result of all the recent studies on parasitology is that puncture by the sting of certain mosquitos is the indispensable condition to malarial infection in man, just as for bovine malaria (Texas fever) inoculation through puncture by the tick is necessary. We shall see how all the projects of sanitation have for their object the removal from malarious regions of the conditions necessary to the life of the diptera which are capable of inoculating man with the germ of malaria.

The prophylaxis of malaria may, therefore, consist (1) in the destruction of the parasite in the outside world, either directly or by removing the conditions necessary to its existence; (2) in preventing the entrance of the parasite into the body of man; (3) in rendering the human organism so resistant that it will be able to impede the development of the infection after the parasite has entered into it.

Destruction of the Parasite in the Outer World.

The malarial germ may be directly destroyed by destroying the insects which contain it ready for the inoculation of man, or by taking away from these insects the conditions needful for their existence. The destruction of the insect hosts of the malarial parasite may be effected in two ways, namely: (1) by the destruction of the eggs, the larvæ, and the nymphæ of the mosquito in marshes, pools, swamps,

and even the smallest collection of water in malarious regions, and (2) by the destruction of the adult insects in and near human habitations.

The destruction of the mosquitos in the stage of their existence which is passed in stagnant water has already been tried in various parts of the world, especially in the United States. Various means are adapted to this end. If fish can be made to live in the water, they will devour the larvæ and nymphæ. When fish will not live there, then a continual movement of the surface of the water by means of a water-wheel put in motion by a wind-mill would suffice to prevent the exit of the adult mosquito from the puparium and also the deposit of eggs, both of which actions require a perfectly smooth surface of the water for their carrying out. According to some, groves of eucalyptus trees in the neighborhood of marshes will keep away mosquitos, and so prevent them from coming to lay their eggs in the stagnant water.

A measure of great value in the destruction of the larvæ and nymphæ of the mosquito is the pouring of petroleum into water which contains them. The determination of the value of this procedure we owe to Aaron, Howard, and Delbœuf. Aaron says that one drop of petroleum will destroy all the larvæ and nymphæ in a puddle ten inches square within fifteen minutes! He proposes the extensive adoption of this measure because it is innocuous, efficacious, easy of execution, and cheap. Howard states that he has succeeded in killing with petroleum all the insects in a pool measuring more than sixty square feet of surface, and says that a barrel of petroleum costing $4.50 would suffice to cover a body of water measuring ninety-six thousand square feet of surface. He adds that the petroleum should be used in the early spring in order to impede most effectually the development of the mosquitos.

Other chemical substances than petroleum have been employed in the destruction of the larvæ and nymphæ of the mosquito contained in stagnant water; among these are sulphate of iron, permanganate of potassium, etc. Celli and Casagrande have recently undertaken the study of this subject, which is one of such practical importance, in the Hygienic Institute at Rome. The results obtained by them will be published soon, and we can only say here that certain aniline colors which are very diffusible and very cheap were found by these experimenters to be endowed with a markedly toxic action as regards the eggs, the larvæ, and even the nymphæ of certain forms of diptera.

The destruction of adult mosquitos near dwellings has been proposed by Aaron and Beutenmüller (quoted by Nuttall). This is

effected by placing at a certain distance from the house lighted lamps on plates containing a little petroleum; the mosquitos are attracted by the lights, and many fall into the oil and perish. We may add the suggestion that these lamps be furnished with powerful reflectors turned away from the house. But the destruction of the mosquitos may be effected more easily within the houses. In all places where these sleep-disturbing diptera are found measures of one kind or another are employed to keep them away at least from the bedrooms. Celli and Casagrande have recently made a comparative study of the action of various culicidal substances, and the results are shown in the following table which they have kindly permitted us to copy.

ACTION OF CULICIDAL SUBSTANCES UPON ADULT MOSQUITOS (CULEX ANULATUS, C. PIPIENS, C. SPATIPALPIS, ANOPHELES CLAVIGER).

Substances Employed.	Period Elapsing Before— Apparent Death.	Actual Death.
I. Odors, Essences.		
1. Nutmeg	10 min.	2 hrs.
2. Camphor	4 to 5 "	4 to 5 "
3. Garlic	5 " 10 "	5 "
4. Powdered pepper	20 "	6 "
5. Naphthalin	10 " 35 "	8 "
6. Sage	.....	Survived.
7. Rosemary	.....	"
8. Dried basil	.....	"
9. Cinnamon	.....	"
II. Smokes.		
1. Tobacco	.....	1 to 3 min.
2. Eucalyptus leaves	3 to 5 min.	3 hrs.
3. Persian insect powder	7 "	3 "
4. Quassia wood	16 "	5 "
5. Pyrethrum powder	5 "	8 "
6. Spearmint leaves	5 "	8 "
7. Tar	10 " 13 "	8 "
8. Dried basil leaves	2 " 6 "	24 "
9. Rosemary	7 " 12 "	24 "
10. Fir-wood	2 "	36 "
11. Chamomile flowers	4 "	36 "
12. Peppergrass leaves	4 "	36 "
13. Sage leaves	8 " 10 "	36 "
14. Firewood	5 " 7 "	12 " 48 "
15. Resin of guaiac	12 "	Survived.
16. Myrrh	15 "	"
17. Gum elemi	15 "	"
18. Incense	15 "	"

III. Gases.

1. Anhydric sulphurous acid		1 min.
2. Sulphuretted hydrogen		1 "
3. Illuminating gas		2 "
4. Formaldehyde (from methylic alcohol)		10 " 15 "
5. Sulphide of carbon	15 to 30 '	Survived.
6. Acetylene		"

If the destruction of mosquitos within dwelling-houses is useful in summer it is especially so in winter. In the autumn, in houses located in malarious regions, the bedrooms, the kitchen, the closets, the cellar, and the attic are all places of refuge of numerous mosquitos, that is to say anopheles, almost all of which are infected. These, continuing to bite the inmates of the house, inoculate them with malarial parasites which will develop later. In the winter the female mosquitos hibernate in these places and from time to time, especially in warmed apartments, may bite man. Now, the destruction of these hibernating mosquitos is doubly useful, because being fecundated females they will deposit their eggs the following spring in water, whence new generations will arise capable of acquiring the infection from, and transmitting it to, man, to say nothing of the possibility of their being inheritors of the infection from the previous generation.

It is, of course, readily understood that the direct destruction of mosquitos cannot be the only means of prophylaxis against malaria; the most important measure is to remove the conditions necessary to the life of the mosquito. To this end, although not premeditated, have been directed all the measures of sanitation undertaken up to the present; and those undertaken in the future will all the more be with that object since they will be guided by the absolute knowledge of what the enemy to be combated is, and where it is concealed.

A review of the history of hygienic improvements shows that all the works undertaken with this end in view have had also the effect of removing from malarious regions the conditions favorable to the life of certain insects, and especially of mosquitos of the genus Anopheles, to the development of which is necessary stagnant water, or, rather, water the surface of which is covered with vegetation. A study of the manner in which a natural sanitation occurs demonstrates the same fact. In the section on Parasitology we spoke of the limited malarial endemic near the city of Senigallia in a small commune on the banks of a canal which served to connect the river Misa with the sea, and to collect the water from the neighboring hills. In summer the water became stagnant in the bottom of the canal and was collected in numerous pools; and the sea entered it only for a short distance, the bed of the canal having become choked up with a deposit of clay.

Now in the autumn of 1897 there were heavy rains and a consequent inundation which swept the bed of the canal free from the accumulations and levelled the inequalities which made possible the formation of pools of stagnant water; and in the following summer the pure water of the sea entered the canal freely and the tide ebbed and flowed through it. Following this change in the condition of the canal the malarial endemic ceased; in the summer and autumn of 1898 there were no cases of malarial fever in that part, not even mild ones, while in the preceding years there had been many cases of malaria, both slight and severe, especially among children. With the fevers disappeared also the mosquitos which in previous years had existed in swarms, troubling the wretched inhabitants of the commune. Now the condition which favored the development of the mosquitos was the stagnation of water in the bed of the canal, the banks of which were covered with vegetation that extended out over the surface of the pools. The new conditions following the dredging of the canal by the floods of water and the cleansing of the banks resulted in the driving away at once of the mosquitos and of the malaria.

But examples of artificial sanitation by the labors of men are more numerous than those of nature's hygienic successes. Examples of this kind, namely, of malarious districts becoming healthy and rich in consequence of hygienic and agricultural works, can be found in all parts of the world. The celebrated work of Francesco Torti on the pernicious fevers was the fruit of observations and studies made in an Italian city where now scarcely any cases of malaria are ever found, and those only in laborers who have been working in certain parts of the surrounding country.

The subject of the sanitation of malarious districts belongs to the province of the sanitary engineer, and we shall touch only upon some of the more important parts of this question which have come up as a result of recent studies.

Of the conditions necessary to the production of malarial endemics certain ones only can be removed. A certain degree of heat is necessary to the development of malaria, that is to say, to the development of the mosquito within the body of which the malarial parasite completes its cycle of existence; and the parasites of the grave malarial fevers require a higher temperature than do those of quartan or tertian fever. But we are, of course, powerless to regulate the temperature of the atmosphere.

In the case of humidity of the soil, stagnant pools, or a slowly moving stream of water, we are often able to accomplish much; for example, the drying up of marshes, ponds, and stagnant pools, and effective

drainage and ditching, and the construction of dikes along the borders of the sea and the banks of rivers are some of the oldest of measures for removing the principal factor of malaria. The drainage of lands where the subsoil contains much water in consequence of the geological structure is a measure employed by many of the peoples of antiquity. The researches of Secchi, Lanciani, Canevari, di Tucci, and Tommasi-Crudeli have shown the existence of an extensive network of canals in the tufaceous hills of the Roman Campagna; and according to the interpretation of some of the writers above mentioned, the object of this vast drainage system was to collect and carry elsewhere the subsoil water of these hills. Tommasi-Crudeli claims to have proved this experimentally, for when the accumulated earth was dredged out from some of these drains the water began to drop from their vault and sides. This drainage and ditching have constituted the main factors in the sanitation of many parts of the world.

The drying up of ponds, pools, and every collection of stagnant water is necessary in the sanitation of a place, because with the disappearance of the stagnant water disappear also the conditions necessary to the development of the insect carriers of the malarial infection. In order to prevent the formation of swamps and stagnant pools the construction of dikes along the seashore and of levees along the banks of rivers and other watercourses is often necessary.

The drying up of marshes is obtained by drainage where there is an incline towards the sea or a running watercourse; but where the land is below the level of the sea, special engines are necessary to raise the water to the outlets emptying into the sea. This system is employed in Holland where extensive tracts of marsh land have been reclaimed from the sea and converted into "polders," that is to say, large basins which are kept dry by pumping engines and given over to agriculture. The sanitation has been undertaken of the marshes of Maccarese and Ostia by this means, and many agriculturists have gone to the latter from other parts of Italy; but there are still many stagnant pools in this "polder," and the malarial endemic is consequently not yet overcome.

The natural filling up of low lands by deposits brought thither by the muddy water of rivers, although a slow process, is productive of much better and more permanent results than those obtained by pumping. Tommasi-Crudeli, so long ago as 1870, proposed that the marshes of Ostia and Maccarese be filled up by the deposits from the muddy waters of the Tiber. Sanitation by the filling process was called by Tommasi-Crudeli "atmospheric sanitation," because he thought that the covering up of malarious soil by mud containing no germs prevented the buried infection from being transported in the

air. He interpreted in the same way the improved sanitary conditions obtained by covering the earth with gravel or pavements. All cities located in malarious districts furnish examples of this mode of sanitation. In Rome the malaria has been driven farther and farther from the centre by the erection of buildings and the construction of paved streets, and now many streets and squares which prior to 1870 were notoriously malarious have lost their evil repute. But the malaria may return with a return to the old conditions, as was shown in the Trastevere quarter of Rome in 1878. At that time the preliminary work for the embankment of the Tiber resulted in the formation of many large pools of water along the right bank of the river, and there developed in consequence a very severe endemic of malaria.

The drying of wet soil has also been attempted by means of plantations of eucalyptus (Eucalyptus globulus, E. rostrata), trees which grow very rapidly and so draw much moisture from the soil, effecting, as Tommasi-Crudeli remarked, an upward drainage. Several instances are alleged of sanitation by means of extensive eucalyptus plantations; some assert that the odor of the leaves kills the germs and also, according to Nuttall, drives away the mosquitos. But the improved sanitary condition is not always the result solely of planting eucalyptus trees. The often cited sanitation of Tre Fontane, a place near Rome, outside the Porta Ostiense, where there is a Trappist convent, and where recently a very extensive plantation of eucalyptus trees was made, especially on the elevations overlooking the sea, is not yet complete. And furthermore, the improvement obtained cannot be attributed to this plantation alone, because other works, such as drainage and intensive cultivation, have also been undertaken. We have said that the sanitation of Tre Fontane is not complete, for in the summer and autumn many of the religious catch fevers and have to go away.

The importance of forests in relation to malaria has been variously regarded. Once it was thought that woods were of especial value in preventing the transportation of the infection in the air blowing from neighboring malarious regions. This opinion, which, sustained by the authority of Lancisi, was long held, has been attacked by Tommasi-Crudeli on the ground of its contradiction by facts. It has been shown that malaria may remain limited to regions of small area, where the conditions are favorable to its existence, without being carried in any direction to neighboring districts.

While it has not been proved that woods serve to hinder the transportation of malaria from infected to healthy regions, the demonstration is also wanting that they serve any useful purpose in the sanitation of a district. On the contrary, there are many examples of

malarious parts which are well wooded and others of improved sanitary conditions following the cutting down of trees. When the groves are on level ground and badly kept, pools of water being formed within them and the dampness being permanent because of the difficult penetration of the sun's rays, we find all the conditions favorable to the development of malaria and the production of malaria-spreading insects. Indeed, the number of insects in such woods in summer is most striking, and both men and animals within them suffer terribly and often emerge actually bleeding from the bites. But while woods in the plain do not protect against malaria, they may, when covering hills and elevations surrounding unhealthy plains, aid indirectly in the sanitation of the latter; and for this reason as well as for others the growth of trees on elevated places should be favored.

Hydraulic sanitation is intimately connected with agricultural, that is, with the cultivation of the drained lands; and for this reason large estates embracing malarious districts should be split up into small holdings because of the intensive cultivation which this promotes. Intensive cultivation, according to Tommasi-Crudeli, should be introduced wherever possible on account of its sanitary as well as its economic advantages, for in order to obtain the latter it is necessary to procure and maintain hydraulic sanitation—the foundation of all agricultural improvement.

Sometimes it is not possible to drain marshes, in which case the bottom of the swamp may be dug out and surrounded by an embankment in such a way as to create a lake. This method of sanitation has been successful in many places, as, for example, in the marshes of Averno and at Lake Fusaro. Father Secchi maintained that equally good results could be obtained by this treatment of the swamps of Ostia and Maccarese, but Tommasi-Crudeli objected to this proposition that the expense of excavating such vast areas of swampy ground would be enormous.

Those who believed that water was the sole vehicle of the malarial parasite contended that the first condition of health in a malarious region was a pure water-supply. But even now, when many observations and experiences, all concordant, have excluded this mode of infection, it will still remain one of the first elements of public hygiene to provide a pure drinking-water, since this offers a safeguard against some other infectious diseases and will aid in preserving good digestion, thus giving to the inhabitants of the malarious districts a greater power of resistance against the prevailing endemic.

Prevention of the Entrance of the Parasite into the Organism.

Up to the present we have occupied ourselves chiefly with the general public prevention of malaria. We shall now devote a few lines to the precautions necessary in order to avoid infection, to prevent the penetration of the malarial organism from without into the human organism, and to the measures which are of service in rendering man immune against infection; that is to say, to the so-called individual prophylaxis.

The very work necessary for the sanitary improvement of a country, and also all other works which call for a turning up of the soil, such as railway construction, the embankment of rivers, the building of fortifications, etc., are often accompanied by an increase of the existing malarial endemic or even by the appearance of the disease in places previously free from it. Examples of this are furnished by the grave endemic which arose during the draining of the marshes of Chartreuse near Bordeaux, the aggravation of the already existent endemic resulting from the digging of the Panama canal, and the endemic just referred to which arose in the Trastevere quarter of Rome, during the construction of the embankments of the Tiber. Such experiences warn us that works of this sort should, when possible, be carried on only during the season when malaria does not occur or when it occurs only in mild form, and not in the summer or early autumn. But now that we know how the infection is acquired it will not be difficult to protect those who are obliged to sojourn and work in malarious regions throughout the year.

The same rule holds good in the case of travel or of the sending of scientific or military expeditions into countries where grave malaria is endemic; the season should be selected when the disease prevails with least intensity. When this is not possible every effort should be made to avoid long halts in the more malarious districts. A model of such an expedition was the English campaign against the Ashantees on the Gold Coast in 1895. The expedition was organized in the month of March, which is the least unhealthy in that region, and all the transportation machinery was in such excellent condition that the English troops tarried only one hour on the coast and immediately entered a road more than sixty miles long, built by the natives, along which were numerous bamboo cabins well stored with everything the sodiers could need. This campaign merited the nickname of the "Doctors' War," as did another campaign, also by the English, in the Guinea coast in 1874, the good health of the soldiers having been

due entirely to the adoption of the measures advocated by the medical staff.

When works of whatever sort must be continued through the unhealthy season, the hours of labor should be so arranged as to begin after sunrise and cease before sunset. During the rest of the time the workmen should remain in elevated places in the vicinity, to and from which they might possibly be conveyed by a temporary tramway or railroad.

It is well known that malarial infection occurs most readily at night, and especially in the twilight when, as Dante says, "la mosca cede alla zanzara." It is an easy matter to remove laborers from specially malarious regions during the most dangerous hours, for just as there may be circumscribed endemics of malaria in healthy localities so there often are salubrious oases in malarious regions. Thus we find districts, such as Norma, Sezze, and others, at a very slight elevation above the Pontine marshes, where there is no danger of catching malaria.

The construction of houses where the laborers may shelter themselves and rest is a necessary undertaking in malarious regions. These houses should be built in elevated localities, exposed to the wind, at a distance from any collection of stagnant water, where the soil is dry. The ground around the houses should be paved or covered with gravel or asphalt, and provisions should be made for running off to a safe distance the drippings from the roof.

Various measures are useful in preventing the entrance of insects, especially blood suctorial insects, into the houses. Among these are the construction of houses with windows opening high above the ground or with those which open only on a paved court; the placing of wire screens in the windows; shutting the windows before sundown; the lighting of fires in front of the houses in the evening; the placing outside of lamps, with reflectors turned away from the houses, in plates containing culicidal solutions; the use of ventilators, etc. All these precautions will be of service also, during the malarial season, in houses on the outskirts of cities lying in unhealthy plains, as, for example, in some of the suburbs of Rome, where we often find persons who have not gone outside of the city, especially if they are so imprudent as to sleep with open windows, attacked by malaria. The employment of mosquito netting is also very efficacious, as is well known, and it is absolutely necessary for those who are obliged to sleep in tents or under the open sky. In the latter case the insects will be much less troublesome if one sleeps on a platform raised on poles some twelve or fifteen feet above the ground, access being had by ladders. This is a precaution which is observed in many mala-

rious regions, as, for example, in the Pontine marches, and in certain parts of Greece where the peasants who are obliged to keep night watch over their flocks or over the ripe grapes pass their vigil on elevated platforms of this sort. In Central and South America, the Indians fasten their hammocks as high up in the trees as possible, and the engineers employed in the construction of the Panama canal built for themselves little wooden huts in the trees where they passed the night.

It is well to remember that anything in which infected mosquitos may be carried may itself become a vehicle of infection, such, for instance, as hay or straw brought from a malarious place. Herein lies the explanation of many isolated cases of malaria occurring in individuals who have not gone outside of healthy cities surrounded by malarious plains.

Those who are obliged to remain out of doors in places where there are many mosquitos may preserve the skin from attack by dusting it with certain powders, bathing it with certain liquids, or smearing it with oils or pomades. Numerous observations would seem to prove that in malarial regions workers in sulphur are relatively immune against infection. According to Abadie (quoted by Nuttall) the elephant hunters in Ethiopia venture down into the open plains with impunity, because, as they assert, they secure immunity against malaria by daily dusting sulphur over the entire body. Petroleum and preparations of tar and other similar substances have a preservative effect against insect stings. Osborn (quoted by Nuttall) says that the employees of the Hudson Bay Company use tar water. Tar is mixed in a large receptacle with oil of turpentine, and upon this is poured water which is allowed to stand for some days when it is ready for use. Oil of eucalyptus also seems to be of considerable value. The following recipe is taken from *Janus* (Nuttall) for 1898: Ether and alcohol, each 5 parts; cologne water and oil of eucalyptus, each 10; tincture of pyrethrum, 15; this mixture is added to water in the proportion of one part to four or five, and the skin is bathed with it. Bathing the exposed parts with an infusion of quassia is also said to be efficacious in preventing mosquito bites.

All these measures of protection against the parasites of malaria are, of course, neglected by the poor, ignorant farm laborers, who in their struggles for bread take little heed of the dangers to which their poorly remunerated labor exposes them. In this case we must resort to a system of legislative sanitary protection—a "social prophylaxis," as Celli calls it—the object of which is so to regulate labor in malarial regions as to secure the best possible protection for the life and

health of the workman. An active and intelligent medical surveillance should be exercised in malarial regions where there are many laborers, with a view not only to prophylaxis, but also to prompt treatment of the sick. This latter is also an indirectly prophylactic measure, since its effect will be to prevent the development of those forms of the malarial parasite which are ready to complete their cycle of existence in the body of the mosquito, and so to restrict the spread of the infection. If this mode of prophylaxis were seriously carried out, we would not see such a slaughter of human beings and such suffering in those desolated malarial regions whither healthy and strong men go in crowds from non-malarial regions, where work is scarce, without thinking of the sad fate that awaits them.

Strengthening the Resisting-Powers of the Organism.

In addition to the prophylactic measures just considered, there are others the object of which is to increase the resisting-power of the organism and so render it capable of preventing the development of the infectious agent which has gained admittance to it. These measures are both hygienic and medicinal.

The hygienic measures consist in food of the proper quality and quantity, good drinking-water, sufficient hours of rest, suitable clothing covering the entire body, etc. As regards alcoholic beverages, we believe that taken in small quantity they are useful and promote the well-being of the laborer; but the abuse of alcoholic drinks is by no means, as some have erroneously believed, a preservative against the malarial infection; for in addition to other reasons alcohol in undue amount is harmful because it predisposes to the cerebral forms of pernicious fever. The English troops campaigning in hot countries are not allowed to indulge in alcoholic beverages, and the result of this prohibition has always been favorable to the health of the soldier (Laveran). Laborers working in the sun during the hot part of the day should not take any alcoholic drink before the evening meal.

But in addition to the adoption of measures for increasing the resisting-powers of the organism, every effort must be made to evade the occasional causes of infection. Excessive labor, especially under exposure to the sun, sudden cooling of the body or bathing during profuse perspiration, sexual excesses, etc., should be avoided as tending to lower the resisting-powers of the organism to infections of every sort.

Among the *medicinal means* for preventing the development of the malarial organism within the body, cinchona bark and quinine have

always held the first place. The use of quinine as a prophylactic has followed naturally upon its employment in a therapeutic sense: if quinine will cure the disease it ought also to prevent it. But this argument is not always valid; another specific remedy, mercury for example, does not certainly prevent the development of syphilis, yet it cures the disease when once established. Nevertheless it is the universal experience of medical men, especially of military surgeons, in all parts of the civilized and uncivilized world, in times of peace and of war, in every malarial country, that very many if not all of these who do not take prophylactic doses of quinine fall victims to malaria, while very few of those who take the remedy suffer from an attack, and those few have the disease in a mild form.

The mode of employment of quinine as a preventive varies. Some give small doses of 0.2-0.3 gm. (gr. iii.–ivss.), others larger amounts; some advise that the drug be taken every day, others give it systematically every three, five, or six days. It is our belief that the method of giving quinine two or three times a week is the most efficacious, just as relapses are surely prevented by doses of quinine given every four, five, or even six days. But the prophylactic doses of quinine, and the frequency of its exhibition should vary according as the prevailing malaria is mild or grave in character and also according to the season of the year. For example, in places where a mild form of malaria prevails, and in the spring of the year in those regions where severe forms of the disease occur only in the summer and autumn, we may begin the administration of quinine on the fourth or sixth day after arrival, and repeat the drug every four or six days in doses of 0.5-1 gm. (gr. viiss.–xv.), given best at bedtime in order to avoid the ringing in the ears and other quinine symptoms during the day. In places where the prevalent form of malaria is severe, as in the tropics, or during the summer and autumn in those districts where the disease is grave only at this time, the first doses of fifteen grains at bedtime may be taken on the third or fourth day after arrival and repeated every fourth day thereafter. The reason for administering quinine in this way is evident. The malarial infection manifests itself after a variable period of incubation, which is, however, certainly not less than five or six days, during which the parasites multiply and finally reach a number sufficient to cause fever. Now, the administration of quinine with a view of impeding the evolution of the parasites in small daily doses will have no such efficacy as that of one large curative dose every four to six days. And, furthermore, we must not forget that the malarial parasites may become habituated to small quantities of quinine in the blood, and can continue their evolution in its presence, as we see in certain chronic cases which

resist small doses of quinine, and even large ones, especially if they are exhibited irregularly.

Some recent observations would seem to indicate that euquinine has the same preventive efficacy as quinine. This, if true, will be of great advantage, for it disturbs the organism less than quinine, and may also be given with ease to children.

But quinine prophylaxis is a specific prophylaxis, and other non-specific tonic remedies have been employed to prevent malarial infection. Among these remedies arsenious acid aroused great hopes after the favorable results of trials made in Italy under the direction of Tommasi-Crudeli. But these trials were interrupted before it was possible to arrive at a positive conclusion. Recently, de Gouvea (mentioned by Laveran) has published certain facts tending to establish the prophylactic efficacy of arsenic.

Serum Immunity.—In order to preserve man living in a malarial region from infection it has been suggested that he may acquire immunity by the same means as those employed to confer immunity against other infectious diseases. We have already spoken, in the section on etiology, of immunity against malaria, but this immunity, whether congenital or acquired, is very rarely seen, even among races which have for ages contended against malaria. Celli and Santori endeavored to confer immunity by injections of blood serum taken from animals that are immune against all forms of malaria even in the most unhealthy regions, such as indigenous cattle, horses, and buffaloes. Results were noted, however, only exceptionally, and then the only effect was a prolongation of the period of incubation. They further tried the blood serum of cattle which had been cured of Texas fever, but they found that neither that nor blood infected with bovine malaria could preserve man from malaria; there is, therefore, no such reciprocity in respect of malaria as there is between variola of cattle and variola of man.

In the further course of their studies, Celli and Santori endeavored to determine whether the principles upon which is founded serum immunity in the case of diseases resulting from bacterial intoxication prevail also in malarial infection. But it could not be positively demonstrated that the blood serum contains a pyretogenous toxin at the onset of the fever, or an antitoxin at the period of defervescence. Therefore neither the blood serum obtained after spontaneous recovery from malaria nor that of immune individuals possesses any immunizing effect, and consequently there is no reason to hope that we can ever obtain a serum immunity against malaria. But more than this, these experimenters conceived the idea that, if the period of incubation was sometimes prolonged after injections of the

serum of animals immune to all forms of malaria, it might be because the blood contained some active principle which was accumulated and stored, as happens with antitoxins, in some special organ. They, therefore, made many attempts to confer artificial immunity by means of the juice of various organs (spleen, bone-marrow, brain, lymphatic glands) of animals absolutely immune from all forms of malaria. But the results of these experiments were neither constant nor positively favorable.

Finally Celli and Santori have made another series of experiments looking towards artificial immunity by means of medicinal substances. The results of these experiments are not yet definitely determined, but we can certainly reject as inefficacious the blood serum of the horse subjected for a long period to intravenous injections of quinine in large and increasing doses. This cinchonized serum is inert as either a prophylactic or a curative remedy in human or in bovine malaria.

TREATMENT.

Spontaneous Recovery.

Before proceeding to the treatment of malaria, it will be advisable to take up the question of spontaneous cure, that is to say, of the recovery which occurs without the use of the specific remedy, by virtue of the natural powers of the organism.

The great majority of diseases due to infection, if cured at all, are cured spontaneously; treatment is only symptomatic, either because a specific remedy does not exist or because we are ignorant of its existence. Spontaneous recovery sometimes occurred in malaria before the efficacy of quinine was known, and of course still takes place when we do not press the administration of the specific remedy.

Spontaneous cure is observed not only in mild fevers, that is to say, the quartan and especially the tertian, but also in fevers of the estivoautumnal group. Its occurrence in pernicious infections is a thing which has never happened in our experience nor in that of many other physicians; indeed, considering that in many cases of perniciosa death has occurred in the first attack, notwithstanding the administration of large doses of quinine, that the same result has been seen in all our cases which were not treated at all or else not treated in time, and that recovery followed in such cases only as were treated with quinine, we are inclined to hold that spontaneous cure of pernicious malaria does not occur, although not positively asserting that such is the case.

Spontaneous cure is accomplished by a gradual diminution of the febrile paroxysms until they finally cease; but it sometimes occurs

after an attack which is graver than any of the preceding ones have been, as to duration, height of the temperature, and the accompanying symptoms. If we endeavor to discover the cause of spontaneous cure, we find that our chief information comes from what a clinical examination teaches us. Patients who have had fever for several days and come to the hospital from field or factory, where they have often gone on working in the intervals of the attacks, are frequently cured after two or three attacks simply by rest in bed, and the ingestion of wholesome and simple food and drink. These are indeed the usual factors of many spontaneous cures even in other infectious diseases, wherefore in general we can say that hygienic measures alone sometimes suffice to awaken in the organism energy and strength sufficient to overcome the cause of the disease and to regain health.

But what are the immediate factors in the spontaneous cure of a malarial infection? Does examination of the blood furnish us with data sufficient for its recognition?

The most obvious factor seen in an examination of the blood is the gradual or sometimes the rapid diminution in the number of the parasites, until only a few are found in the last attacks or attack. Sometimes there are many mature parasites or those approaching maturity near the time of an attack, while during the attack itself the young parasites are so few that it often takes a long time to find them. But it may also happen that the cycle of generation represented by a few parasites continues to be observed without the occurrence of any corresponding febrile attack.

While diminution in the number of parasites is taking place, certain changes may be observed in some of them which may lead them to be regarded as degenerative and dead forms; that is to say, they are mature bodies, pigmented at the centre, vacuolated, and about to disintegrate; and young pigmented forms free in the plasma, which we may suppose to have escaped from the red corpuscles and to be no longer endowed with vitality. In tertian fever, the spherical bodies, larger than a red blood cell, with mobile pigment, vacuolated, and disintegrating readily under the microscope, were considered by Celli and Antolisei to be parasitic forms which, while in process of multiplication, were set free in the plasma, degenerated, and died, and they believed the presence of these forms to be connected with the occurrence of a spontaneous cure. Bignami and Bastianelli noted that in tertian fevers which were cured spontaneously, and especially when the recovery occurred after a severe attack, these forms were present in much greater abundance than in cases which did not tend towards recovery. The same thing was noted for the similar bodies described

as occurring in the quartan fevers; indeed, in some cases they were able to ascertain that when these special forms increased the young endoglobular parasites did not appear later.

What is the cause of the death of the parasites in process of development, and whence it proceeds is as yet unknown. Mannaberg, having observed degenerated and fragmented forms of tertian and quartan parasites during the febrile attacks, believes that the febrile process exercises a destructive action upon the parasites. But in opposition to this view is the fact of frequent malarial infections which become progressively more severe, although accompanied with high fever. As in all infections so in the malarial, in order to explain the phases of increase, of status, and of decrease presented by the febrile affections in which recovery takes place spontaneously, especially in the relapses, it has been supposed that the parasite in the human body acquires, preserves, and even exalts, and then loses its virulence. In harmony with this diminution in the virulence of the parasite is the fact that in the period of decrease there may be a succession of two or more generations of parasites without any corresponding febrile attacks. The weakening of the parasites finally ends in their death, as shown by their disappearance from the circulating blood.

We must bear in mind, however, that in spontaneous cures the degenerated and dead parasites are not constant in the peripheral blood, nor when present are they found in such numbers as to be of use in the prognosis, enabling us to predict recovery. Their destruction is likely to occur by preference in the internal organs and chiefly in the spleen and the bone marrow; moreover, if they are young parasites, which usually die in the plasma before the period of invasion of the red blood corpuscles, a microscopical examination of the blood will not enable us to recognize anything except the gradual or the rapid weakening of the new generations up to their total disappearance.

Whether the exhaustion in virulence of the parasites, which has such disastrous consequence to themselves and such beneficial ones to the human organism, is inherent in the parasites or is a result of the defensive powers of the body, as for instance in the production of some substance capable of killing the free spores in the plasma, is a question which future investigations will have to determine.

Phagocytosis, which as we have seen is accomplished in every variety of malarial infection by some of the leucocytes and some of the endothelial cells of the blood-vessels of certain viscera, has been considered to be a factor in the destruction of the malarial parasite. When the process of phagocytosis has been observed under the micro-

scope, that is to say, when we have been able to see the leucocytes approach the parasites, enfold them in their prolongations, introduce them into their protoplasm, and gradually disintegrate them until nothing is left of them but a small mass of pigment; when we have seen the phagocytes containing not only pigment, but free and endoglobular sporulations, young parasites within apparently normal red corpuscles, and numerous spores, we have almost been persuaded of the truth of the hypothesis that phagocytosis represents a defensive measure of the organism against malaria, at least to the extent of keeping the multiplication of the parasites within certain limits, for as they become the prey of the phagocytes they are subject to destruction. Some of the spores are sometimes preserved; although a direct proof of their survival cannot be given, an indirect one is seen, according to Bignami, in the later relapses, which are caused by the wakening to activity of spores which have been latent for a more or less long time. But of this and of other things relating to phagocytosis we have already spoken. There remains to be seen whether phagocytosis is more active in spontaneous cure, as would seem to be the natural inference from the view that they are a factor in the defensive power of the organism. Now our own observations and those of others (Bastianelli) have not given constant results. In some cases with the attenuation of the attacks there has seemed to be an increase in the phagocytes in respect to the number observed on the days preceding those in which the infection showed a tendency to become extinguished; in other cases the gradual disappearance of the parasites appeared to be accompanied by a diminution in the number of the pigmented leucocytes. We must also note that, if there are free spores in the plasma where they die, their presence among the white cells easily escapes us. But even if it were found that in cases of spontaneous recovery there was always an augmented number of phagocytes containing parasites and pigment, there would remain to be seen whether this proceeded from primary increase in the activity of the phagocytes or from the fact that the parasites, by defective vitality without manifest morphological change, became an easy prey to the phagocytes, thus stimulating the functional powers of the latter. We must therefore conclude that as yet it has not been fully demonstrated that phagocytosis has any special rôle in the effecting of spontaneous cures. It is, however, certain that the phagocytes contribute to the result by purifying the blood and the organs of detritus which if allowed to accumulate would certainly interfere with their proper functions.

Another fact which it has been often sought to connect with spontaneous cure deserves mention; this is the appearance of those spe-

cial parasitic forms which in the estivoautumnal fevers are represented by the crescent bodies that are so often seen to become flagellated, and in the quartan and tertian fevers by the large spherical bodies which may also become flagellated. In the estivoautumnal fevers the diminution and spontaneous cessation of the attacks not infrequently coincide with the appearance of crescent forms in variable number, generally corresponding to that of the preceding parasitic forms. These crescents and other allied forms persist in the blood for several days even when recovery is quite complete. Whatever may be the nature of this class of bodies, it is certain that they do not belong to a pyrogenous cycle, and that in the human body they go on, without multiplying themselves, to degeneration and death. And therefore, in the human body, the formation of these bodies after those of the pyrogenous cycle have disappeared signifies the conversion of a fever-producing infection into one which is not fever-producing, the attenuation of the infection, and frequently the approach of its extinction.

With the exception of these scattered data furnished by an examination of the blood—the death of the parasites in the various phases of their life cycle especially of the free spores in the plasma, the development of parasitic forms destined to complete their cycle in another organism, and phagocytosis, we know of no other occurrences which could be deemed factors in spontaneous recovery, unless, indeed, we were to enter into the field of theories. We must bear in mind the fact of spontaneous cure and its relative frequency in some forms of the infection, whenever we wish to judge impartially of the effect of new remedies or of new methods proposed for the cure of malarial infection.

Let us now see whether any practical information can be gathered from the ideas held upon spontaneous recovery. The older physicians (limiting the term to those of a time posterior to the discovery of the specific action of quinine), who well recognized the fact of spontaneous cure of the intermittent fevers in the spring season, especially the tertian, either employed purely hygienic treatment or prescribed mild laxatives, leaving a cure viribus naturæ. The distinguished clinician Torti often recommends that winter intermittent fevers be left "viribus naturæ et imminenti veri." But the same physicians, including Torti, inculcated the necessity of immediate treatment with cinchona bark in autumnal tertian and in malignant fevers. In following their example we shall run no risk provided we do not neglect to give quinine, not only in grave fevers, but also in mild ones when after two or three attacks the hygienic treatment does not seem to produce a diminution of the attacks nor of the parasites. But since

recovery ensues more easily and more rapidly when the specific remedy is given, expectation of a spontaneous cure is justifiable only in case the patient were to derive from it the benefit of immunity against relapses. Now experience teaches us that relapses occur after spontaneous recovery and even after a succession of spontaneous recoveries, such as sometimes take place especially in the winter relapses. And when these successive relapses are left to a spontaneous cure, is an immunity from reinfection acquired, as in scarlatina, for instance? Not in the least; indeed, the sad experience of every day demonstrates the fact that after a long series of relapses which finally end in the spring, a return to a malarial region will frequently bring a return of the fever. Therefore, in our climate at least, spontaneous recovery does not confer complete immunity against either relapses or new infections. The most that is acquired is a resistance to progressive infection (pernicious), but not an immunity sufficient to prevent relapses and reinfections with all their train of consequences up to a state of chronic infection and cachexia. Yet in some persons there is an immunity against the infection, acquired after they have had one or more attacks a year for many years. Thus in the Roman Campagna, in the region of severe malaria, we not infrequently see in the midst of the general squalor, healthy and robust individuals of flourishing appearance, who state that they have suffered from the fever in the past, but do so no longer, although living in the same place and under the same conditions as those who frequently succumb to the infection. But these immunes do not all recover spontaneously—indeed, the majority of them state that they were cured by quinine. Moreover, they for the most part belong to a people who for centuries have been fighting malaria, such as those living in the unhealthy regions of the Pontine marshes and of the Roman Campagna. But of absolute and relative immunity, hereditary or belonging to the race or individual, congenital or acquired, we have already spoken, and if we have here returned to the subject, it has been with the sole purpose of demonstrating that permanent immunity against malarial infection is not acquired even when spontaneous recovery has occurred several times, and that even in mild cases, except under very exceptional circumstances, a delay in the administration of the specific remedy is not advisable.

Mode of Action of Quinine.

Malaria is one of the few infectious diseases which possess a specific remedy. This, as we all know, is cinchona bark, the great virtue of which is due to its alkaloid, quinine ($C_{20}H_{24}N_2O_2$), the salts of which are at present used in malarial infections almost to the exclusion of other remedies.

The history of the discovery of this invaluable remedy, and of its introduction and diffusion throughout Europe and the other parts of the world, is so well known and so fully described in all works on pharmacology that it would be superfluous to relate it. What is of more especial interest to us is a knowledge of the mode of action of quinine in malaria, and the reason of its almost prodigious efficacy in virtue of which so many lives have already been saved, and will continue to be saved until some way shall have been discovered of killing the malarial agent in the external world, or of preserving the human organism from its action.

Upon the mode of action of cinchona and its chief alkaloid, quinine, in malarial infections, especially upon the way in which it causes the cessation of the intermittent fever, many theories have been held in the past, the majority of which testify to the ingenuity of those who originated them no less than to the erroneous impressions under which they labored, as shown by the light of recent discoveries regarding the etiology of malaria.

According to some physicians, who believed that intermittent fever proceeded from lesions of the nervous system, quinine acted upon the nerves and the nerve centres, inhibiting or interfering with the exhibition of the physiopathological activities whence emanated the fever. Others, endowed with greater perspicacity, who held that intermittent fevers were the result of a ferment or a miasm or some parasitic agent, thought that quinine acted against the primary cause of malaria by neutralizing or destroying it. Among the physicians of this second category are to be mentioned Francesco Torti and Binz. The former, who early in 1700 gave instruction as to the dosage and the manner of administration by which cinchona could be rendered most effective against pernicious fevers, which up to that time had been regarded as inevitably fatal, believed that when quinine in sufficient degree of concentration met in the blood the pyrogenous ferment which from time to time was poured out into it, causing the intermittent fever, it destroyed its injurious powers. Cinchona bark, said Torti, acts not "in febrem, sed in solam causam febris."

Binz, believing that the cause of intermittent fevers was of a parasitic nature, held that quinine acted upon this agent as a poison, basing his views chiefly upon the fact that quinine is a poison to many species of infusoria.

Recent investigations, made since the discovery of the malarial parasite, have been directed towards ascertaining what became of the parasites, by following all the changes which they underwent after the administration of quinine given at various times during the periods of fever and of apyrexia, and therefore during the various phases

in the life cycle of the parasites themselves. In these researches the action of the remedy upon the course of the fever both before and during an attack, as well as in successive attacks and relapses, has been carefully noted.

It is known that the salts of quinine are absorbed by the blood, and eliminated in great part by the urine. According to the experiments of Binz, Kerner, and Lepidi-Chioti, this elimination begins in from fifteen to seventeen minutes after ingestion by the mouth, twenty to twenty-five after injection into the rectum, ten to fifteen after hypodermic injection, and ten after intravenous injection (Baccelli). According to Kerner all the quinine is eliminated in from thirty-six to forty-eight hours. A part passes from the blood into the bile (Albertoni).

The action of quinine has been studied upon all the known species of parasites and at the same time in relation to the various febrile types. Antolisei, Golgi, Romanowski, Mannaberg, Ziemann, and others studied the influence exerted by quinine upon the amœbæ of the tertian and quartan forms of malarial infection. According to Antolisei, the adult bodies of the quartan are not hindered in their development by quinine; sporulation occurs as usual, and so does the febrile attack, which is at most delayed for one or two hours, but the attack which should follow is lacking. In the attack occurring after the administration of quinine, only a very few endoglobular forms are found. "The fission-forms disappeared from the blood as in other attacks, but in contradistinction to the latter there was no substitution by the usual number of non-pigmented bodies—rather by a much-diminished quantity; they evidently during the present attack felt the deleterious action of the quinine, and only a part of them were able to escape from it" (Antolisei).

The most complete researches made upon this subject, however, are those of Golgi. According to his researches in cases of quartan fever, the administration of quinine in ordinary therapeutic doses does not arrest the development of the parasites when the modifications preceding segmentation have begun. The febrile attack is not prevented, yet the young generation of parasites resulting from fission is destroyed, and this accounts for the permanent cure obtained with only one dose of the remedy three to four hours before the attack. If quinine is given six to eight hours before the attack, when the parasites are mature, but before the process of segmentation has begun, the attack is delayed and attenuated. But if the quinine is given on the first day of apyrexia, when there are only young endoglobular bodies present, its action is not constant. Sometimes the expected attack does not come because the parasites have been de-

must remember that in the estivoautumnal fevers the thermic and the parasitic resistance may vary notably in each separate case. At all events, in the majority of cases the results, as we have already said, vary with the time of administration of the medicine.

In order to render what we have to say more clear, we will briefly note the dose and the manner of giving the remedy adopted in our experiments. The amount is that which is usually administered in our hospitals in cases of estivoautumnal fever. The first prescription is rarely less than 2 gm. (gr. xxx.) of the sulphate or the bimuriate of quinine, usually taken in two equal portions with an interval of from two to four hours. This initial dose is generally followed every twelve hours by smaller ones. This is the treatment in cases of ordinary gravity. In cases which are grave but are not accompanied by pernicious symptoms, the initial dose is usually 1 or 2 gm. injected hypodermically. In the pernicious fevers, hypodermic injections are always given, starting with full doses of from 2 to 3 gm., followed by smaller doses, usually of a gram, at intervals of from four to six hours. The following are the results obtained:

If the quinine is administered during the crisis of an attack and continued during the apyrexia which follows, in the majority of cases the next expected attack is inhibited or there is merely a slight elevation of temperature with a slight sense of discomfort. In a smaller number of cases, even when strong doses of quinine are given during the crisis and the period of apyrexia, the attack is not prevented, but is delayed and abortive, that is to say, composed of one elevation of temperature alone and that only a little above 39° C. (102.2° F.), or else is simply abortive though not retarded. When there is delay, it may be slight, or it may be of twenty-four hours. In rare cases no influence whatsoever is manifested upon the following attack, but nearly always a third attack is prevented. If the dose of the remedy has been small (as 1 gm. only by hypodermic injection), not only does the attack next in order appear, but a third one follows it, and this one may act in various ways. It may present the typical curve, or it may be abortive, or else prolonged. In some of our cases these attacks which followed the administration of quinine often showed more pronounced oscillations of temperature, and the interval of apyrexia was more decided and longer than usual. The return of a second and even of a third attack may be observed in rare cases, even when the doses of quinine have been large and repeated (as for instance, 2 gm. and more every twenty-four hours), and the new attack may also be prolonged (see chart No. 17). In tertian fevers with a tendency to subcontinuity, quinine administered in the same manner may result in complete apyrexia following the attack; but it

does not prevent the return of other attacks, although it may modify them in the manner noted. For a description of the modifications produced by quinine in the form and in the succession of the febrile attacks, see the clinical histories and the charts appended in a previous section.

If quinine in the usual dose is administered within the six hours preceding the expected attack it may have no influence at all upon the

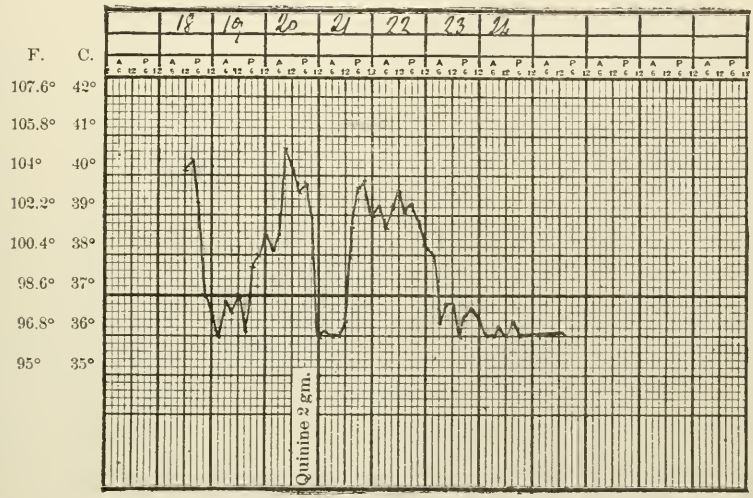

CHART No. 17.—Case of Pietro Pacifici. Estivoautumnal fever, with a prolonged paroxysm following the administration of about 2 gm. (grs. xxx.) of quinine.

temperature of the succeeding paroxysm; in other cases there is noticed a slight delay in the attack which also is less severe than the preceding one, but even in this case the temperature curve is the typical one of an estival tertian. Subsequent attacks as a rule do not occur.

If the remedy is administered at the onset of the attack in the majority of cases the attack will come on in the usual way and may even be grave and prolonged; very often, however, there are some modifications in the curve—either an exaggeration of the pseudocrisis so that the attack tends to lose its individuality and almost to become duplicate, or a rapid crisis occurring after the initial elevation so that the attack is markedly shortened. But these modifications are apt to occur when the dose of the remedy has been large and given hypodermically. A subsequent attack does not usually occur, although there may be frequent irregular elevations of temperature.

When the quinine is given during the febrile attack, beginning shortly after the onset and continuing throughout the course of the fever, in a series of cases the characteristic curve of the tertian is not appre-

ciably modified, while in another series there are various modifications—attenuation or disappearance of the precritical elevation or exaggeration of the pseudocrisis, or it may sometimes happen that the attack is prolonged and lasts longer than did the preceding one. In a whole series of cases, when the quinine has been given in large amount during the attack, there are apt to be no subsequent attacks, or on the following day or days there are only slight elevations of temperature.

In pernicious tertian, or in pernicious infections of tertian origin with irregular fever, it is not possible to analyze the events as we are able to do in mild fevers and in those of medium gravity, because hypodermic injections are given at the very beginning of the observation and continued at short intervals. Now in one series of cases the temperature will be promptly lowered, even when the pernicious symptoms persist, and in another the fever will persist in spite of the energetic administration of the remedy, and there may even be hyperpyrexia for a few days until death finally occurs. Between these extremes there are intermediate cases, in which, notwithstanding the continued use of quinine, the pernicious attack may for several days be followed by febrile attacks which are for the most part abortive.

From a comparison of these data with those of the fever in the ordinary tertian and the quartan, we can see that there is no constant relation between the gravity of the infection and the resistance of the fever to the action of the remedy.

Although in the quartan and the ordinary tertian fevers we succeed better in avoiding relapses by the administration of quinine a few hours before the attack, the same cannot be said of the summer-autumn fevers, in which, according to our experience, relapses occur as a rule; in some cases indeed it is impossible to avert them, no matter what the method of administration of the quinine, even when it is continued for several days in succession. And not only are we unable to avoid a recurrence of the febrile paroxysms, but we may, although rarely, after an interval of apyrexia lasting four or five days, have a relapse, and a grave one, in spite of repeated large doses of quinine.

Effects of Quinine upon the Parasites.

The explanation of the above-described facts is found, of course, in the action which quinine exercises upon the life of the parasite. Let us now see what is this action upon the estival tertian parasites, by studying their various phases, and then administering the remedy during the various periods corresponding to the development of the febrile attacks, and in the doses of which we have already spoken.

If quinine is given near the time of the crisis, when the blood contains

only young non-pigmented parasites, and if its administration is continued for about twelve hours, the parasites continue to be seen in the peripheral blood for nearly twenty-four hours, together with pigmented leucocytes; then they disappear without undergoing any further development, while the red corpuscles which contain them present those alterations which have earned for them the title of "brassy bodies"; the disappearance of the amœbæ may in some rare cases be more prompt, being completed even in twelve hours. If quinine is given in the same way at the onset, but its administration is suspended after a few hours, it may happen that the young amœbæ persist in the blood for more than twenty-four hours (thirty-six or even forty-eight hours) and then develop, becoming pigmented very tardily, so producing a delayed attack. In exceptional cases, even when the dose of the remedy has been large, no influence is noted on the development of the parasite, which then gives rise to a new generation.

If quinine is given in the last hours preceding the attack, when the only bodies found in the blood or the predominant bodies there are pigmented adult parasites, then the parasites go on with their development up to fission, but the new generation as a rule does not present itself in the following attack, which, being usually abortive, leads to the supposition that not all the parasites have reached complete maturity. In other cases, while we do not see the generation of young parasites, we have bodies belonging to the crescent group; it would appear that in this case the parasites, instead of going on to sporulation, take the other road which leads to the formation of crescent forms.

When the remedy is given at the beginning of the attack, at the time when in the blood we find fission forms or those that have already become divided (although these are very rarely seen in the peripheral blood at this time, but are certainly found in the internal organs), the action of the remedy is subsequently recognized by the fact that the amœbæ of the new generation become extremely scarce, and if the quinine be continued, they disappear entirely from the blood within twenty-four hours, while the red corpuscles which contain them become brassy. In the young amœbæ there are absolutely no signs of development. In spite of the scarcity of the parasites during the attack, this may be grave as to duration, the height of the fever, and the accompanying symptoms. There are even some cases in which, although the salts of quinine are given at the beginning of the attack and continued throughout its course, the attack is prolonged. In such a case, a careful examination will show that the new generation of amœbæ appears many hours (sometimes twenty-four) after the beginning of the fever, and in scanty amount. It is

probable that the reason of the prolongation of the attack is found in the delayed entrance of the amœbæ within the red blood corpuscles.

The facts which we have described are confirmed by studies made in those cases in which in the blood we find *parasites in various stages of development*, when it is possible in the same blood to follow the effect of quinine upon the different phases in the existence of the parasite. This is what we see: if in the blood there are non-pigmented parasites and adult forms about to multiply, as occurs in some grave fevers, the adult forms disappear entirely from the blood, and after a period of time which may vary from twenty-four to forty-eight hours, the young forms disappear after the red corpuscles containing them have become brassy; the pigmented leucocytes persist in the blood even longer than the parasites. This result is obtained by continuing to give the remedy in three or four doses at intervals of from six to twelve hours.

Even in the gravest infections and in fatal pernicious fevers we may observe the same progressive diminution and successive disappearance of the parasites, following repeated strong doses of quinine, up to the time of death. It is easy to see, from what has been said, that among the cases of grave infection there are some which suggest a peculiar resistance to the action of quinine on the part of the parasite. It thus sometimes happens that in spite of the administration of the remedy some of the young generation will continue to develop until they cause a new attack which may be prolonged and even fatal. The new colony of parasites may be so scanty as to escape detection for several hours, but later the endoglobular forms gradually increase in number during the attack, this increase being to a certain degree in correspondence to its gravity. In these cases also, if the infection has lasted several days, the appearance or the increase in numbers of the crescent forms will correspond to the diminution in number of the young endoglobular bodies.

In quotidian fevers practically the same facts are observed, namely, when quinine is given after the crisis of an attack, the young forms persist for a somewhat lengthened period in the blood, even more than twenty-four hours, and then disappear without having shown any indication of further growth. If the remedy has been given in insufficient doses or even in one large dose (2 gm. hypodermically, for instance), the development of the young amœbæ may still occur. When quinine has been given a little before the beginning of the attack, or just at the beginning, the attack is not averted, but the invasion of the red corpuscles by the new generation is prevented.

The salts of quinine, even when given in doses as large as 2 gm. a day, do not perceptibly affect the crescent forms of these

parasites, as all have observed who have interested themselves in these experiments.

Having now seen how the parasites in the blood are modified by the action of quinine, let us examine more closely the morphological and biological alterations that this alkaloid produces in the summer-autumn parasite, having already spoken of what takes place in the quartan and the tertian according to Golgi's observations. The young endoglobular amœbæ are not seen to undergo any change for several hours after the administration of quinine; their motility persists and in some cases is very active. After a few hours the amœbæ take on a discoid shape, and some are seen to be in process of making their exit or detaching themselves from the red corpuscles. The observations made in specimens of blood stained according to Romanowsky's method do not permit us clearly to distinguish differences between the parasites of patients who have been treated with quinine and those who have not. We have recently had occasion to observe some blood rich in young endoglobular parasites several hours after the administration of no less than 6 gm. (gr. xc.) of quinine without being able to find any alteration in the structure of the parasites. But the biological changes in the parasites are greater than the morphological, and consist in an arrest in the processes of nutrition. The amœbæ without pigment do not take on any, and therefore do not receive nourishment, and in those in which pigment exists, it does not increase, so that growth is prevented. A proof that the amœbæ are incapable of development although they may be morphologically unchanged is found in the fact that blood rich in parasites, but strongly impregnated with quinine, is incapable of reproducing the infection when injected into the veins of a healthy person.

The final fate of the young amœbæ is their death either within the red corpuscles which have become brassy, or in the plasma after they have become free. The latter occurrence is recognized in fresh blood preparations, where we may see young parasites in the act of leaving the red corpuscles, and even better in preparations stained by Romanovsky's method, where, owing to the peculiarity of the coloration, we see the young amœbæ free among the red corpuscles. If the young parasites abandon the red corpuscles before the latter have become brassy, these corpuscles may possibly survive, and this shows the double advantage in the use of quinine. The possibility becomes a probability in those cases of pernicious fever, in patients anæmic from previous malarial infection and in whom there are many young parasites, in which the proper administration of quinine brings about a cure without any increase in the anæmia.

But although young parasites do not continue to develop under

the influence of quinine, if the changes preceding fission have already begun fission will occur in spite of the presence of quinine in the blood. This is evidenced, not so much by an examination of the peripheral blood as by the presence of fission forms in the capillaries of the brain and the other organs, when death has occurred several hours or even a day or so after the administration of large doses of quinine. As a rule, the young parasites resulting from fission do not invade the red corpuscles. From the above facts it is seen that in estivo-autumnal fevers, as in quartan and tertian, the chief and the most rapid action of the remedy is exercised upon the phase of the extra-globular life of the parasite which follows complete sporulation. As to the other life phases of the plasmodium, we may say that the action of quinine is to interfere with nourishment and development, but that the remedy is incapable of arresting the process of fission if this have already begun.

The various data resulting from a study of the action of quinine upon the malarial parasite may be summed up in a general law expressed as follows: *Quinine acts upon the malarial parasites in that phase of their life in which they are nourished and develop. When the nutritive activities cease by an arrest of the transformation of hæmoglobin into black pigment, and the reproductive phase begins, then quinine is ineffectual in its action.*

This resistance of the adult forms in process of fission, which goes on to completion in spite of the action of the medicament, explains the inefficacy of quinine in so many cases of pernicious fever. If fission occurs, no matter what amount of quinine is given and no matter how it is administered, and if during the fission of the parasites and the disintegration of the red corpuscles pyrogenous toxic substances are formed, it is evident that therapy cannot prevent their injurious action, nor, what is of still greater importance, can it prevent the accumulation of parasite-infected red corpuscles in the capillaries of the various organs, especially the brain. In the cases of fatal pernicious fever seen by us, we noted at the autopsy the great predominance of adult forms, that is to say, those with blocks of central pigment, and of fission forms, more especially in an examination of the internal organs. And this, as we have already remarked, is one of the reasons why in a pernicious fever the presence of many adult forms makes the prognosis more serious.

By what mechanism does quinine interfere with the processes of nutrition and therefore with the further development of the young endoglobular parasites, and, although incapable of preventing the multiplication of mature forms, destroy the new generation before it invades the red corpuscles? Are the young endoglobular parasites

killed directly by quinine, or indirectly by its action on the medium in which they live? We know, from the experiments of Binz and of his pupils, that quinine exercises a directly inhibitive action upon the processes of oxidation, and also upon some of those which are accomplished by means of hæmoglobin. The giving up of the oxygen from ozonized turpentine to guaiac which hæmoglobin has the property of causing, does not occur, or is very evidently delayed, if a neutral salt of quinine be added to the blood, although no spectroscopic modification occurs in the hæmoglobin. Rossbach explains this action by saying that, as quinine does not modify the property of hæmoglobin to carry oxygen, the latter combines more strongly with the hæmoglobin and does not so easily pass on to other substances. Now if we thoroughly realize this fact, we cannot avoid the conclusion that the action of quinine is not only exerted directly upon the parasite, but also modifies the red corpuscles in such a way as to render them less or not at all adapted to the further development of the invading parasite.

As to the second fact, namely, that of the non-appearance of the new generation of amœbæ, although fission of the mature forms has occurred, we may suppose that they have been directly killed by the action of quinine free in the plasma, even though some of them have not been born dead or inactive. But we are also at liberty to suppose that quinine produces some modifications in the red corpuscles which indispose them to the harboring of parasites, and these, being unable to go on with their development in a free state in the blood, die there after a while. In favor of this second supposition is the fact of the tardy appearance of the new parasitic generation in such prolonged attacks as follow the administration of quinine. But in either case, whether quinine directly kills the spores which are free in the plasma or causes their death by preventing them from entering the red corpuscles, we must admit that a certain number of spores, possessing a greater power of resistance, escape the destructive action of the alkaloid, or else do not die in the plasma. In consequence of this they form a starting-point for the relapses, which, especially in estivoautumnal infections often occur after a new period of incubation, even after energetic treatment with quinine. Even admitting this exception and whatever its explanation may be, the fact remains that in summer-autumn fevers, as well as in quartan and tertian, the greatest and most rapid action of the remedy is upon the very young parasites resulting from fission, when they are free in the plasma.

Were we to push our inquiries so far as to try to ascertain why quinine is powerless to arrest the process of fission of the parasites, once it has begun, no reply could be expected from an observation of

the morphological data. One view only might be held, namely, that this resistance on the part of the parasitic bodies to the action of quinine comes under the general law governing and ensuring the multiplication of living beings, no matter to what category they belong, even in the midst of the greatest dangers. It is true that, according to the observations of Golgi, Romanowsky, Mannaberg, and others, although the multiplication of the parasites occurs, it does not do so with regularity. But the observations which we ourselves have made, in preparations obtained several hours after death and stained with hæmatoxylon and eosin, on the sporulating bodies of estivoautumnal parasites in the capillaries of the brain and other organs in cases of pernicious fever, after treatment with large doses of quinine, have not demonstrated any structural differences between these forms and the ones found in fatal pernicious fever when there has been no specific treatment or when treatment has been begun only a short time before death.

Knowing the part taken by phagocytosis in malarial infections, it remains to be seen whether this function of the leucocytes has anything to do with a cure obtained by means of quinine. From the investigations of Golgi upon phagocytosis in quartan and tertian fevers, it is evident that this occurs in the same manner in the blood of those who have been treated with quinine as it does in those who have not taken the remedy, with the sole exception that the process may be somewhat delayed by the action of the quinine. Golgi draws the conclusion from this that phagocytosis plays no part in the cure of malaria by quinine, the drug acting directly upon the parasites. In estivoautumnal infections we have often seen the number of phagocytes notably increased after the administration of quinine, especially in pernicious affections, and as frequently and to the same extent in cases which went on to recovery as in those which ended fatally; but this does not seem to us to be due to an increase in the energy of the phagocytes by direct action of the quinine upon the leucocytes, but to an increase in the number of dead parasites and in free pigment, following the toxic action of quinine. The phagocytes increase in number because the cadavers of parasites and of red corpuscles and the masses of pigment increase, and the blood needs to be purified from them. Therefore although phagocytosis cannot be regarded as a factor in the cure resulting from the administration of the remedy, still its activity in the blood of patients who have taken quinine is not to be denied. An entirely different conclusion would have been reached from a study of the observations of Binz and others, which tended to demonstrate that the action of quinine paralyzes the leucocytes, which therefore become powerless to accomplish those amœ-

boid movements which are so essential to the process of phagocytosis. But this paralyzing action of quinine on the leucocytes has not been confirmed by other observers (Hayem quoted by Laveran, Disselhorst), nor by the researches which we ourselves have made in malarial infection. In order to account for the fact that in the presence of quinine the diapedesis of the leucocytes does not occur, although they are not deprived of their motility, Metchnikoff holds that there must be a phenomenon of negative chemotaxis by means of which the leucocytes are kept from going to the tissues impregnated with quinine.

Rules for the Administration of Quinine.

From the above description of the studies which have been made upon the action which quinine exerts on the malarial parasite, the marvellous efficacy of this remedy is seen to consist in its being a poison to the pyrogenous parasites. The confirmation and *rationale* of the rules for the administration of quinine, already practised by many physicians and universally recognized as of value, are also clearly demonstrated. As early as the beginning of the eighteenth century Torti inculcated the precept that in ordinary intermittent fevers cinchona bark was to be administered "hora ingruentis paroxysmi," and that in cases of double tertian it should preferably be given before the gravest attack, because he knew that by giving the remedy before the light attack this would be suppressed, but the other attacks would continue, and thus the double tertian would be converted into a simple tertian. To prevent relapses, Torti gave a small dose of cinchona daily for the eight days following the cessation of the fever; he then suspended the treatment for several days, administering the drug again for six days, and so on for a variable length of time, all this being, as he said, "for the purpose of destroying the residuum of the ferment which caused the relapses." Sydenham held the same view, with this difference, that he gave at intervals of a week and in one dose the same amount of quinine which had served to overcome the fever. In pernicious fevers Torti gave a triple dose of the remedy, or even more, beginning as far in advance as possible of the attack, and if this was imminent or had already commenced, he advised that the drug be given generously in two or three doses at short intervals, or even in one dose, not so much for the purpose of influencing the attack which had already commenced as to prevent the next attack and the fatal issue which would certainly occur without the drug—if indeed it did not take place during the attack which it was impossible to avert. Puccinotti, who studied intermittent fevers in Rome in the first half of this century, held that in all miasmatic

intermittent fevers the largest dose of the specific should be given nearest to the onset of the fever; that to abstain from giving the quinine up to that time could and should be done in intermittent tertian and quartan of benign nature; and that the older the fever and the more rebellious it had proved to small and divided doses, the more necessary was it to carry out this plan. The explanation of the greater efficacy of quinine when given just before the attack is to be found in the fact that its action is displayed most powerfully when the young parasites are free in the blood, and therefore are more easily affected by the remedy.

Taking into account both the experience of the older scientists and the results of the investigations carried on since the discovery of the parasite of malaria, we are now in a position to give the rules as to time and amount which should be followed in the administration of quinine.

In the double tertian, and in the triple and even in the double quartan, the indications are properly met when quinine is given in the dose of 1 to 1.2 gm. (gr. xv.–xviii.) during the period of apyrexia, in such a manner that the largest dose shall be administered three or four hours before the attack. The same dose is to be repeated for the two or three days following, and in order to avoid relapses it should be repeated every six days for a month or more.

In the simple tertian and quartan, and also in the double quartan, especially in recent infections, it will be useful to give the same dose of quinine at the end of the attack, and to repeat it six or four hours previous to the hour at which the other attacks usually began. The first administration at the end of the attack is justified by the knowledge of the fact that quinine also acts upon the young endoglobular parasites, especially those of the tertian; and that, when the drug is given during this period of the life cycle of the parasites, although it may not prevent the attack, it will at least make it milder, shorter, and even abortive in its type. This is an advantage not to be despised. The further treatment of simple and double quartan, and of simple tertian is the same as that of the more complex types.

In chronic tertian and quartan infections, especially when quinine has already been given in insufficient doses and in an irregular manner, it will be well to suspend the use of the remedy for a few days, and then to administer it in the dose mentioned before the expected attack. An examination of the blood will be of use by indicating the opportune time for the administration of the remedy.

In estivoautumnal fevers, whether tertian or quotidian, if the symptoms or the condition of the parasites do not demand an immediate administration of quinine, it should be given towards the end

of the attack in a large dose—1.5 or even 2 gm., the same dose to be repeated for three or four consecutive days. Since the periods of apyrexia interposed between the attacks in the summer-autumn tertian and quotidian are short, the first administration of quinine should be during the few hours preceding the expected attack. The administration of the medicine on the succeeding days is for the purpose of preventing further attacks; and to prevent relapses the same treatment is followed as in the other types of fever, that is to say, a dose of 1.2 gm. of quinine is given at least every six days for several weeks.

In fevers with pernicious symptoms, and in cases in which the condition of the parasites gives warning of a pernicious attack, quinine should be immediately given in a 2 gm. (gr. xxx.) dose by hypodermic injection (in the other fevers, unless the condition of the digestive tract forbid, it is administered by the mouth). This initial dose is followed at intervals of from six to eight hours by doses of 1 gm. (gr. xv.) or less, according to the course of the fever. Subsequent treatment directed to the prevention of relapses is the same as in the other forms of the infection.

It has been held that we should wait until the fever subsides before giving quinine; but although that may be proper in the simple fevers, in pernicious infections the remedy should be given just as soon as possible, for to await the period of apyrexia, especially in prolonged attacks and in subcontinuous forms, would be to run the chance of being too late.

We have said that in order to prevent relapses an appropriate dose of quinine should be given every six days for a period of several weeks. To this interrupted method of administration some prefer the giving of small daily doses for a longer or shorter time. Experience, however, has proved the first method to be the more effective, and the explanation is not far to seek. Relapses, as we know, are due to the fact that the parasites which have survived spontaneous recovery, or a cure due to quinine, continue to multiply until they become sufficiently numerous to produce the fever. Now small daily doses of 20 or 25 cgm. (gr. 3 or 3¼) will not prevent the relapse, just as they will not check an attack; while a large dose given at intervals of several days, and preferably within a few hours of, or at the time when the attack used to appear, will interrupt the development of the parasitic cycle which is preparing to bring about another attack. Indeed, it is presumable that about the same amount of quinine in the blood is necessary to arrest the life of the parasites, whether these be few in number or whether there be enough of them to cause fever.

Quinine then should be administered at the right time and in

proper doses taken within a brief period; at the right time because its greatest activities are then developed just when the parasites are the most vulnerable; in a certain dosage and all within a brief period, because if the amount is insufficient, or if it is sufficient but is broken up into small doses taken at too long intervals, we shall not obtain the concentration of the remedy in the blood necessary to the destruction of the parasite. Moreover, when quinine is taken without regularity in insufficient doses and at long intervals, the fever becomes irregular, and even rebellious to regular treatment instituted later, as if the parasites had become used to the action of quinine. This is seen in malarial country places, where the poor laboring people, partly on account of their poverty, are accustomed to take quinine in this fashion. Were it given properly and in time, under strict medical guidance, we should not have to deplore the loss of so many lives from pernicious fever in malarial regions, nor the ruin of so many from protracted fevers.

Of the salts of quinine, the hydrochlorate and the sulphate are the ones most used. The former is the more soluble and contains the greater proportion of quinine; the latter is easily rendered soluble by the addition of a few drops of sulphuric acid.

The methods of administration of quinine vary. In simple intermittent fevers the salts of quinine are given by the mouth, either in solution or in very thin wafers. In the latter case the ingestion of the quinine is followed by a draught of hydrochloric-acid lemonade. In the case of babies, to whom it is difficult to give quinine by the mouth, an attempt may be made to administer it in an enema, doubling or tripling the dose, and adding to the solution of quinine a few drops of the tincture of opium, regulating the amount according to the age of the child. If the remedy is tolerated neither by the mouth nor by the rectum, or if there are special conditions which prevent the choice of either of these methods, and the fever shows no sign of abatement, then hypodermic injections will have to be resorted to. The same course is to be followed in grave and pernicious fevers.

We now use *hypodermic injections* with greater frequency than of old, because we know that the injuries resulting therefrom are more often than not the result of a neglect of those precautionary measures of asepsis which should always be followed in regard to the solution, the instrument, and the technique of the operation. The solution adopted for hypodermic injections in our hospitals is the following: Quinine hydrochlorate, 5 gm. (gr. lxxv.), distilled water q. s. ad 10 c.c. (℥ iiss.). By using this solution we know that with a Pravaz syringe we inject 50 cgm. (gr. viiss.) of the salt. We can also use more dilute solutions, as 1:2 or 1:3, in order to render the injec-

tion less injurious. The solution should be clear, and the instrument and the skin of the part where the injection is to be given should be rendered aseptic. The injection may be made in the back, abdomen, or gluteal region, in which situations some prefer to give it intramuscularly as being less painful or entirely painless. Although more or less troublesome subcutaneous indurations are sometimes unavoidable, severe eschars, abscesses, and even worse local injuries can be avoided by observing the precautions mentioned above; strict asepsis will also prevent the occurrence of tetanus, which in former times not infrequently made its appearance, usually a few days after the injection, following inflammation at the point of puncture.

Recently Baccelli has suggested a method of *intravenous injection* of quinine when prompt and energetic treatment is necessary. Such injections are indicated in those cases of pernicious fever in which an examination of the blood shows the presence of many parasites, particularly if they are on the point of fission, which indicates the approaching loading of the blood with parasites; or in pernicious attacks already at an advanced stage in which, because of cardiac weakness, we have reason to fear that absorption from the tissues would be slow. In algid pernicious fevers, whose fatality is well known, when the peripheral circulation is reduced to a minimum, it seems to us that the intravenous injection of quinine ought especially to be tried. The formula of the solution to be used, according to Baccelli, is the following: Hydrochlorate of quinine, 1 gm. (gr. xv.); chloride of sodium, 0.75 gm. (about gr. xii.); distilled water, q.s. ad 10 c.c. (℥ iiss.). The solution must be perfectly clear, and should be tepid. Before giving the injection a bandage is bound above the elbow, as in the case of blood-letting, in order to swell the veins of the forearm; in one of these veins the needle is introduced, and then, the bandage having been removed, the fluid is slowly pushed in. A swelling at the point of injection is a sign that the needle has not been properly introduced into the vein. The dose which will secure the best results is that of a gram, although the minimum efficient dose may be less than that. The dose of a gram, as Baccelli observes, corresponds to 1 part in 5,000 in the blood mass, or, in other words, to the solution of quinine which Binz deemed sufficient to kill the protozoa. Baccelli has seen a cure follow in all cases of pernicious fever treated in this way; after twenty-four hours the parasites had all disappeared, there was no return of the fever, nor were there relapses. Many physicians in the hospitals of Rome and of other malarial regions have tried the efficacy of intravenous injections of quinine in very grave cases of pernicious fever. But not all the cases end in recovery; there are several on record at the Santo Spirito Hospital in which

death occurred, in spite of this treatment, about twenty-four hours after it was instituted—that is to say, when the quinine introduced directly into the vein had had ample time to exert its influence. This fact should not cause surprise, because we know that in a few rare cases the patient may die even after the total disappearance of the parasites, and we also know that there are parasitic infections in which, after the continuous administration of large doses of quinine, we find at the autopsy parasites in the viscera in every phase of their existence. This proves that there are some incurable alterations of the organism secondary to a parasitic invasion, although not always visible at the autopsy, and that there are also parasitic infections which appear to have a special resistance to quinine in whatever way this is given.

Quinine Poisoning.

All physicians, especially those practising in malarial regions, are aware of the fact that the salts of quinine, so useful in the treatment of malaria, sometimes act, even in medicinal doses, as a poison to the organism, producing symptoms which vary as to form and gravity.

We do not refer to the ringing in the ears, the deafness, the trembling, the slight confusion of mind, and the discomfort complained of by nearly every patient who takes the remedy in the doses ordinarily prescribed in malarial fever; but of other symptoms far more troublesome, often grave, and sometimes even threatening to life. Deafness, amaurosis, psychical excitement (quinine inebriation), uneasiness, labored breathing and even dyspnœa, cutaneous eruptions, especially urticaria, partial, diffuse, and even scarlatiniform erythema, hemorrhages into the skin, symptoms of cardiac weakness even to collapse, intestinal colic and enterorrhagia, uterine colic and metrorrhagia, etc., are the most frequent poisonous symptoms following therapeutic doses of the drug. Of the toxic symptoms following doses larger than the medicinal, and taken by mistake or for purposes of suicide, or from mistaken zeal, we have no need to speak in this connection.

The occurrence of these injurious symptoms after a medicinal dose of quinine is rather rare; they are the result of an intolerance or of an idiosyncrasy in certain persons, which is usually congenital, and is observed every time that quinine is given. The patients are well aware of their own peculiarity in this direction, and inform the physician of it. We recall the case of a woman who always had uterine colic and metrorrhagia after the taking of quinine, and one of a youth who was always profoundly prostrated and sometimes swooned after taking the drug.

The physician ought to be aware of all the possible injurious effects of quinine, in order that he may profit by the knowledge in his practice, and above all, that he may not attribute to an aggravation of the disease symptoms which are the effect of the treatment.

The duration of these symptoms varies, but usually they disappear in from twenty-four to forty-eight hours after the administration of quinine has been suspended; but they may last several days, as happens in the case of cutaneous eruptions, intestinal disturbances, and disturbances of sight or hearing. As to amaurosis, it may persist for weeks and months, and even when the sight has been restored there is usually some diminution in visual acuteness with concentric narrowing of the visual field.

Among the injurious effects which sometimes follow the therapeutic administration of quinine, we have not yet mentioned *icterohæmaturic fever* or *hæmoglobinuria from quinine*, because we have thought it better to treat briefly of this affection by itself as a form of quinine intoxication which demands the attention of all physicians, since it is often of more serious import than the disease which is under treatment, yet is frequently considered to be only an effect of this disease.

It is a singular fact that this form of quinine intoxication has long been known to people living in tropical climates, so that they fear quinine more than they do malaria, while the inexperienced physicians believe that their ideas about it are a mere prejudice, and continue to administer the drug, in the belief that in this remedy is to be found the only means of cutting short the disease, and even of saving the patients.

The credit of having directed attention to this form of poisoning belongs to Tomaselli.* His observations were confirmed by Karamitzas, Theophanidis, Pampoukis, Chomatianos, and others, and more recently by the brothers Plehn, Murri, Grocco, Vincenzi, and Bastianelli, besides many French physicians. Laveran holds that icterohæmaturic fever produced by quinine, such as Tomaselli described, has never been demonstrated and indeed he claims that all physicians are agreed in its non-recognition. He then adds that the opinion that such a fever is produced by the action of quinine is a popular belief among the creoles of Réunion Island, but one which is not shared by their physicians who have always protested against the prejudice. But it seems that the *prejudice* was on the part of the physicians, and

* All the writings of Professor Tomaselli upon this important subject, from the first one published in 1874 to the present time, as well as the writings of his pupils, are collected together in his latest work, "La intossicazione chininica e la infezione malarica," Catania, 1897.

that the laity were in the right! Koch, from the observations which he made in the German colonies of East Africa upon blackwater fever, concludes that the majority of cases of this disease are due to quinine intoxication, or when this drug has not been given, to other toxic substances having the same hæmolytic action. Out of 16 cases studied in the Hospital of Daressalem, in 14 there was no malarial infection, in 2 malarial parasites were found in the blood, but the conditions were such that it was possible to exclude malaria as a cause of the hæmoglobinuria. Of 43 cases of hæmoglobinuric fever observed by F. Plehn, 24 followed the administration of quinine, and the attacks developed in correspondence with the period of greatest activity of the remedy; of 53 cases seen by A. Plehn, 48 were the result of quinine intoxication. Hæmoglobinuric fever was *always* observed in persons who were suffering from malaria or who had had it recently, and in both cases the infection had usually lasted some time, and had produced enlargement of the spleen and liver. In the cases of hæmoglobinuria from quinine during malarial infection, as well as in those of postmalarial hæmoglobinuria, quinine had previously been given to advantage, and without any injurious effect.

The dose of quinine necessary to the production of hæmoglobinuria may be less than the ordinary therapeutic dose—0.50, 0.20, and even 0.10 and 0.05 gm. (gr. $7\frac{1}{2}$ to as low as gr. $\frac{3}{4}$) having been known to cause the symptoms referred to. All the salts of quinine without exception, even decoctions and extracts of cinchona bark, in whatever way administered, even by friction (Tomaselli), may produce hæmoglobinuria.

When quinine, administered during the malarial infection, exerts a toxic action, it does not lose its anti-malarial properties, for, the hæmoglobinuria having passed, if the dose of the remedy was sufficiently large, the febrile attacks will stop.

Now since quinine intoxication manifested by hæmoglobinuria does not occur in all chronic sufferers from malaria, nor even in all cachectics, but only in a very small proportion of both, we must admit a special predisposition or *idiosyncrasy* on the part of these malarial patients, which at a given moment is brought into action by quinine. Such a predisposition is often found in several members of the same family, and may even be hereditary, as was noted by Tomaselli and later by Vincenzi. The latter tells of a physician whose mother died from quinine intoxication, who after having suffered from malaria had hæmoglobinuria every time that he took even small doses of quinine.

Quinine hæmoglobinuria in persons who have suffered or are suffering from malaria is not seen with the same frequency in all

climates. In some malarial regions within the tropics it is most frequent, as in West Africa and in German East Africa, in Guadeloupe, and elsewhere. In Europe it is frequent in Greece, not rare in Sicily, and not very rare in Sardinia. In the Roman Campagna, and therefore in the hospitals of Rome, it is rarely seen. In the rest of Central Italy and in Northern Italy the only case known is the one published by Murri.

The symptoms and the course of an attack of quinine hæmoglobinuria have been so accurately described by many observers that it will not be difficult to give a *résumé* of them. After the administration of quinine, given either for the purpose of combating the infection made manifest by the presence of parasites in the blood or to prevent relapses or to cure other diseases which have made their appearance after the malarial infection ceased, a few minutes or hours (from one to six) elapse, then the patient has tremors, a sensation of cold, a severe chill, pain in the epigastric region, in the limbs, and in the lumbar region, pallor of the face with cyanosis of the lips, nausea, the vomiting of bile-stained matter, and severe headache; the temperature may then rise to 41.5° or 42° C. (106.7° to 107.6° F.); the urine is red, dark red, or black; the pulse becomes rapid, very weak, sometimes imperceptible; the respiration is rapid and superficial; after a few hours more or less jaundice appears. The attack usually lasts from twenty-four to forty-eight hours, and leaves the patient jaundiced, anæmic, and prostrated. If we withdraw the quinine and the malarial infection has been overcome, the results of the poisoning quickly disappear, and recovery is not long delayed. But there may, although rarely, be a fatal ending, especially when the physician blindly insists upon continuing the quinine. Death occurs either during the attack with the symptoms of collapse, or after one or two days with symptoms of uræmia. In sixteen cases of hæmoglobinuria reported by Tomaselli, three were fatal; the same number of deaths occurred in sixteen cases seen in Africa by Koch.

The color of the urine varies from reddish to blackish; the quantity is about normal. In the cases with a tendency to anuria, the amount is diminished from the beginning; the reaction is acid, neutral, or even alkaline. The density is in relation to the quantity, but may be diminished even when the amount is normal. The urine contains albumin, hæmoglobin, and bile pigment. A microscopical examination shows masses of hæmoglobin granules, blood casts, epithelial and hyaline casts, renal epithelium stained with hæmoglobin, and sometimes red blood corpuscles. When the hæmoglobin has disappeared, the bile pigment and the albumin persist for a while longer.

The anatomico-pathological examination shows in addition to the lesions of an active or previous malarial infection a jaundiced condition of the organs and lesions of the kidneys, in which, if death have occurred from uræmia after a period of anuria, we find the tubules occluded by casts of coagulated hæmoglobin. A case observed by Bastianelli and Bignami was that of relapse of a grave infection. The first attack of the relapse, on September 3d, was light and was not treated; the second attack, on September 4th and 5th, being more severe and having lasted thirty-eight hours, 2 gm. of quinine by the mouth were given at the end of it. On the morning of the 6th, the examination of the blood giving warning of another attack, quinine was again administered; at 4 P.M. there was an attack of hæmoglobinuria which lasted until 5 P.M. of the 7th, and terminated in death. At the autopsy, in addition to the signs of malaria were found kidneys of normal size, with hyperæmic glomeruli and very brown, fine striæ along the pyramids. A microscopical examination showed degenerative lesions of the epithelium, some of which contained granules of hæmoglobin, and in the cortical substance, but still more in the pyramidal, the tubules were filled with hæmoglobin casts.

Intoxication from quinine in malarial patients is not always so grave as that just described; sometimes there is simply hæmoglobinuria or hæmaturia, as Tomaselli states, without fever, or hæmoglobinuric fever without jaundice.

We have already said that hæmoglobinuria from quinine can occur not only during the existence of a malarial infection, but also after this has been some time absent. The first well-studied case of postmalarial hæmoglobinuria from quinine was reported by Murri. A young girl, seventeen years old, contracted tertian fever in July, 1893, while harvesting in the valley of Comacchio. In the month of January, 1894, the relapses still continuing, she had a first attack of hæmoglobinuria after having taken quinine. After this time up to June, 1894, when she came to the clinic of Professor Murri, whenever the fever recurred and she took quinine she would have an attack of hæmoglobinuria. Then on the 15th, 17th, and 20th of the same month the patient had hæmoglobinuric attacks without having taken quinine, and without any parasites being found in the blood (post-infection hæmoglobinuria). From August 3d, 1894, to April 6th, 1895, small doses of quinine were given eight times to the patient, who no longer had fever, but was constantly improving, and there were eight attacks of hæmoglobinuria. From the description of the first attack, which the others exactly resembled, it is seen that the patient had fever, headache, enlargement of the spleen and liver, and jaundice. The fever lasted about eleven hours, but the icterus per-

sisted for several days. Examination of the urine showed hydruria, alkaline reaction, serum-albumin, hæmoglobin, and in the sediment a few renal morphological elements. In the blood, one and three-quarter hours after the administration of quinine, the serum was normal; after seven and three-quarter hours the serum was of a reddish color and gave the characteristic bands of hæmoglobin. The chief diminution of the red corpuscles occurred on the fourth day after the attack. From April, 1895, to March, 1897, the young girl had no malaria nor any other disease, when on the 27th of the latter month a final experiment was tried by giving her, as before, 0.5 gm. of quinine hydrochlorate in order to ascertain whether there was still any predisposition to poisoning by this drug. The result of this experiment was a fever of short duration, preceded by chills and by epigastric pain, and accompanied by headache, thirst, nausea, the vomiting of biliary matters and mucus, hyperæmia of the conjunctivæ, pain and slight swelling of the liver; the urine was at first slightly acid in reaction, and then neutral, with diminished specific gravity; then, during the course of the attack, the reaction again became acid, and there was gradual increase of the specific gravity, then there followed albuminuria, peptonuria, propeptonuria, and urobilinuria, but no hæmoglobinuria. From the result obtained in this last experiment, Murri holds that the predisposition to quinine poisoning is not a property indelibly fixed upon the organism by malaria, but one of those temporary biological occurrences which gradually become attenuated as the lapse of time removes the organism from the influence of the infectious agent.

Should malarial infection after a long interval again invade the organism which has been thus affected by quinine, the medicament will be efficacious and may be tolerated; but only temporarily, because if the infection persist and the remedy be repeatedly administered, the toxic phenomena will not long delay their appearance (Tomaselli).

Hæmoglobinuria from quinine may also occur during malarial infection in an *inconstant manner*, as has been shown by the observations of Bastianelli and Bignami in Rome, and the Plehn brothers in Kamerun. As an example we will cite a case reported by A. Plehn and quoted also by Bastianelli:

A man living in Kamerun, cachectic for nine months, had suffered from fever for several days. On the morning of March 22d, he took 1 gm. of quinine; in the afternoon he had an attack of hæmoglobinuria from which he recovered. On March 31st there was another attack of fever which lasted until the 7th of April, with a daily elevation of temperature to 40° C. (104° F.); phenocoll was given without result. On May 7th 1 gm. of quinine was injected hypodermically, causing cessation of the fever without hæmoglobinuria. On May

25th, not feeling well, he took 1 gm. of quinine as a precautionary measure. Three hours later an attack of hæmoglobinuria came on and ended fatally.

Finally, the predisposition to hæmoglobinuria from quinine may persist for many years and perhaps for life without the intervention of reinfection by malaria, as we find from observations by Vincenzi and others. Vincenzi cites the case of an officer who, after having suffered fifteen years from malaria and during that time having had attacks of hæmoglobinuria from quinine, preserved his intolerance to the remedy which, whenever he took it even in small doses, and whether he was sick or well, always gave him an attack of hæmoglobinuria. In this, and in other similar cases, the modification caused in the organism by malaria, by means of which quinine always acts as a poison, is indelible, while in other cases it is merely temporary. We see the same thing, as Murri observed, in those beneficent modifications determined in the organism by certain infectious diseases which impart immunity either temporarily or for life.

From what has been said the conclusion may be drawn that quinine, even in very small doses, acts as a poison on certain organisms, whether they now have or have had malaria; but for the most part only after malarial infection has lasted some time and after quinine has been used to good purpose without injury, showing that a certain amount of time is required before malaria can produce the modifications in the organism which render the quinine toxic. The various theories regarding the pathogenesis in these cases are discussed at length in the section on Hæmoglobinuria, and need not be entered upon here.

Succedanea of Quinine.

Since this great remedy was found capable of causing so much harm, it goes without saying that much thought has been spent upon the possible means of avoiding the danger. This result can be reached only by finding some way of rendering quinine innocuous or by the discovery of some succedaneum of equal efficacy. Opium and ergotin have been used to counteract the injurious action of quinine, but to no avail; so that Tomaselli, dissatisfied with the experiments so far attempted, advises that others be made with a view of reaching the desired goal.

The efforts to find a drug to replace quinine have not been any more successful, although many substances have been proposed as succedanea. Of these we shall mention only the chief.

As we know, other alkaloids besides quinine are found in cinchona bark; of these, *cinchonine*, *quinidine*, and *cinchonidine*, especially the

last two, have been tried, but without good results. The sulphate of cinchonine has been used by many physicians in the French colonies (Laveran), but has now been abandoned. The sulphate of cinchonidine, also much used by the French physicians, although useful in light infections, has been recognized as non-efficacious in grave infections (Laveran).

Euquinine, a derivative of quinine, and properly the ethylcarbonic ether of quinine, has recently been the subject of experimentation. According to v. Noorden, a dose of 1 or even of 2 gm. is well supported by the patients, who complain only of a ringing in the ears and a sense of heaviness in the head. Panegrossi experimented with euquinine in eight cases of malarial infection, and always with good results. But further experiments must be made with this remedy in all the forms of malarial infection, and we must, moreover, ascertain whether it is free from the injurious action of quinine. Recently Archangeli has found that euquinine is efficacious against estivoautumnal fevers. The dose should be double that of the salts of quinine. It is very useful in the case of infants.

Phenocoll, which is a body of the aromatic series, was for a long while in use, especially in Italy, and its action upon malaria was studied by Albertoni, Cervello, Novi, Casati, Venturini, Crescimanno, Ancona, Pucci, Materazzo, Vincenzi, and many others. The hydrochlorate was used in the dose of 1 or 1.5 gm. (gr. xv. to xxiiss.) in the twenty-four hours. Some have considered it useful in quartan and ordinary tertian fevers, but not in the estivoautumnal; others believe it to be inefficacious in all kinds of malarial infection. In a communication of Colasanti and Geronzi, it is stated that the latter used the hydrochlorate of phenocoll in twelve cases of tertian and quartan fever, in doses of from 1 to 4 gm. in the twenty-four hours. In eight cases it had no action on the course of the fever nor on the development of the malarial parasite; in four cases the fever ceased and the parasites disappeared after the administration of the remedy. Now in these last cases the scarcity of the parasites and the condition of the patients do not allow us to exclude the possibility of spontaneous recovery; but in the other cases, which for several days had resisted the action of phenocoll, recovery speedily ensued when quinine was used. In the estivoautumnal fevers no good results were obtained. F. Plehn is opposed to the use of phenocoll, because while he never had certain results from its administration, he frequently saw injurious consequences, such as erythematous and bullous eruptions, etc., and he also noted symptoms of collapse when it was given in the dose of 4 gm. in the twenty-four hours. This

same author also quotes Möwe, who in German East Africa observed symptoms of collapse and hæmoglobinuria (?) following the same dose of the remedy.

Methylene blue was first recommended by Ehrlich and Guttmann, who were led to study its action in malarial infection by the observations of Celli and Guarnieri upon the possibility of staining living malarial parasites by this substance. The dose of the remedy is from 0.5 to 1 gm. in the twenty-four hours. Although some experimenters have reported good result from the use of this drug, others have reported the contrary. Thus Laveran tried it several times without success; Mya asserts that of nine malarial patients treated with methylene blue only three were cured, neither was it possible to say that their recovery was not spontaneous. Bein found the parasites in the blood of a patient who had been taking this substance for fifteen days, and for twelve of them in doses of 1 gm. a day. And lately Ziemann, who has given methylene blue in cases which showed no tendency to spontaneous recovery, has become convinced of the complete inefficacy of the drug. Besides the drawback of its staining the urine and later the vomited matters blue, it also produces strangury, and according to Gaillard (quoted by Laveran), in doses of 0.40 to 0.60 gm. (gr. vi. to ix.) it may, although rarely when the substance is pure, cause nausea, vomiting, diarrhœa, and temporary albuminuria.

The preparations of *helianthus annuus* (infusion of the flowers and leaves, tincture of the bark and of the flowers, in doses of a wineglassful three times a day) have recently been successfully tried in Russia, according to Romanowsky. But in patients treated with this remedy, Romanowsky found no morphological alterations in the parasites, but only changes in their typical development; the regular development of the new generations did not occur, and the febrile attacks stopped. But even after the fever had ceased parasites were found, and sporulating bodies were seen every day, even in cases of simple tertian. After the lapse of a few days, the regular development of the parasites was reëstablished, they apparently having got accustomed to the drug, and the febrile attacks began again.

Preparations of *lemon* (juice of the fruit, decoction of the seeds, powdered bark of the root, decoction of the fruit) are a popular remedy against malarial infection, especially of the chronic type, in many parts of Italy and Greece. Tommasi-Crudeli and several who followed his advice to try the effect of lemon in obstinate cases of malarial fever, have observed pronounced and beneficial results.

We omit the description of other substances which have been proposed as succedanea for quinine, as the results obtained by their use by the various investigators have been extremely contradictory, and

the conclusion to which we are forced is that the only remedy deserving the name of a specific in malarial infections is quinine. In order that it should really be such, however, it must be administered rationally—that is to say, at the opportune moment, in sufficient amount, and for a certain length of time, if we wish to attain our end of cutting short the infection and preventing relapses.

Symptomatic Treatment.

According to the opinion of many physicians in the past, the treatment of intermittent fevers was to be commenced by the administration of a purgative, and in robust subjects by both that and bleeding. This practice justly fell into disuse. Yet in light cases of malaria, when there are symptoms of gastric trouble, it will be well to give a laxative and at the same time prescribe a simple, wholesome diet, and rest. Not infrequently under this treatment alone a spontaneous cure will take place, especially if the infection is a recent one and the patient is robust.

During the attacks, the symptomatic treatment is of the simplest description. If the chill is prolonged and very severe, hot drinks should be given; in the hot stage, cold acidulated drinks will be very refreshing; in the sweating stage the perspiration should not be checked, as it may help to eliminate toxins. If vomiting be troublesome, cracked ice may be given; in case of headache cold applications are to be made to the head.

In pernicious fevers symptomatic treatment is often of assistance to the specific treatment. It is unnecessary to state that it must vary according to the form of the disease. In the soporous or comatose cerebral forms in robust subjects, with injected face and conjunctivæ, and a hard, full, and infrequent pulse, many physicians advise that leeches be applied to the mastoid processes to prevent meningeal inflammation. From all that we know as to the pathology of malaria, it is easy to recognize the fallacy of such treatment. In pernicious fevers accompanied by grave delirium and agitation, hypodermic injections of morphine are indicated. If a very high temperature has already been prolonged for several hours and shows no tendency to fall, a tepid bath will be of service. In pernicious fevers, when symptoms of cardiac weakness appear, especially if the temperature is low, we should give stimulating treatment (injections of caffeine, camphorated oil, etc.). But stimulating treatment finds its chief indication in the algid forms; here, in addition to the stimulating injections mentioned, energetic friction of the whole body should be given, the patient should be wrapped in warm flannel, sinapisms are to be

applied to various parts of the body, especially in the cardiac region, and hot stimulating drinks must be administered. The same treatment may be followed in choleraic, dysenteric, and cardialgic pernicious fevers, in which the diarrhœa and the pain should be checked by the use of opium, a hypodermic injection of morphine being given in the cardialgic form. In these last-named varieties of pernicious fever, especially in the choleraic, hypodermoclysis with a physiological salt solution may be urgently indicated. In cases of perniciosa with many parasites and grave anæmia, such as are found in relapses, especially in the autumn, in addition to the specific treatment blood transfusion by Ziemssen's method may prevent death from acute anæmia, should it not occur from the infection.

When quinine has brought about a cessation of the fever and disappearance of the pyrogenous parasites, rest, nourishing food, stomachic tonics if the appetite is poor, and iron and arsenic if the anæmia is marked, will serve to repair the organism of the convalescent; while the interrupted, periodical use of quinine will serve to avert relapses of the infection. Removal to a non-malarial region, and preferably to a high altitude, will be of the greatest benefit. Unfortunately this treatment cannot be followed by all, and least of all by those who are the most exposed to malaria.

Chronic Malaria.

We have seen that in chronic malaria the fundamental phenomenon is the repetition of the febrile attacks, whence the progressive anæmia and the chronic enlargement of spleen and liver. Now to prevent this repetition of relapses, the rational use of quinine accompanied by a hygienic regimen, such as has already been described, will be of almost certain efficacy. We should bear in mind that when the attacks of fever are irregular and the patient is taking quinine in insufficient and irregular doses, it will be advisable to suspend the treatment for several days; and then, after having observed the course of the fever and made an examination of the blood, to give quinine again in sufficiently large doses shortly before the attack, and repeated for from four to six days in succession; after which it should be given in the same dose once every six to eight days. Sometimes in spite of this treatment, especially in the winter, the attacks will be repeated, but as a rule, they become gradually less and less severe until they finally disappear.

In chronic infections when, after cessation of the fever, there remains marked enlargement of the spleen and liver, treatment with certain mineral waters is recommended (in Italy the waters of Montecatini, Chianciano, etc.; in Germany, Carlsbad; in France, Vichy)

for the purpose of modifying the abdominal plethora, of curing the gastroenteric catarrh which is often present, accompanied by constipation or diarrhœa, and of thus obtaining a more rapid diminution in the size of the hypochondriac organs.

In cachectic conditions treatment directed on the one hand to obtaining a cessation of the fever if this still persist, and on the other to the fortifying of the organism, especially if conducted in a non-malarial and healthy climate, is often followed by recovery, provided the incurable alterations already described have not become established. Remedies which stimulate the appetite (condurango, nux vomica, a small amount of alkaline water in the morning, etc.), iron, and arsenic, the latter by hypodermic injection if not tolerated by the stomach, and with these remedies, nourishing food, rest from fatiguing work, the avoidance of any cause whatsoever which might induce a relapse, when the infection is not altogether extinct, and the avoidance of all possibility of fresh infection—these are the principal rules which should direct the treatment of malarial cachexia. As an antimalarial and excellent reconstituent in chronic malarial infection and cachexia, Baccelli recommends the following mixture:

℞ Quinine sulphate,	3. (gr. xlv.)
Iron and potassium tartrate,	7. (gr. cv.)
Fowler's solution,	gtt. xxiv.
Distilled water,	300. (℥ x.)

One to three teaspoonfuls of this mixture are taken daily. If well tolerated it will be of great use, the more so that this preparation of iron does not cause constipation.

Sequelæ.

The treatment of the morbid sequelæ (nephritis, neuroses, etc.) is the same as that of the diseases themselves; but whatever the form may be, there is always the possibility of malarial relapses and an anæmic condition of the patient.

The *enlarged spleen*, even when voluminous, becomes gradually reduced in size when the fever stops. But there are some enormous splenic tumors which, when the fever disappears, become only slightly smaller by very slow degrees, and are the cause of much annoyance and danger to the patient, as has been shown in the section on Sequelæ. To diminish the size of the spleen, we must resort to revulsives, electricity, cold douches over the splenic region, intraparenchymatous injections of quinine, ergotin, liquor arsenicalis, resorcin, etc. The reduction of the organ following these injections is not due to the specific action of the substances injected, as the experi-

mental researches of Murri have shown, but simply to the irritative local action, for the same results are obtainable by the injection of distilled water or by the capillary puncture used by Feletti. The formation of areas of necrosis follows both this procedure and injections, and these are replaced by connective tissue, whence the retraction of the spleen.

In the case of great enlargement of the spleen, especially when the organ is movable or ectopic, surgical intervention has sometimes been considered necessary. This is in truth indicated when there are grave and permanent functional troubles and disturbances, such as pain, a sensation of painful stretching or straining when walking, nausea, vomiting, and abdominal colic—these last in cases of movable spleen probably come from torsion of the pedicle (Tricomi). These and other sufferings are the hardest to bear and the most painful in persons who are obliged to work for a living.

Splenopexy has been proposed by Rydygier in cases of movable spleen, but this operation can be performed only when the spleen is slightly or not at all enlarged.

Splenectomy has of late years frequently been performed in cases of malarial enlargement of a spleen either normally situated or movable or ectopic. In Tricomi's work we find that in cases of chronic malarial enlargement without displacement, there have been forty-six splenectomies with twenty-six cures; for ectopic malarial spleen, thirteen splenectomies with eleven cures; and for ectopic malarial spleen with torsion of the pedicle, four splenectomies, all fatal. After extirpation of the spleen the pains cease, the blood crasis improves, there is a gain in weight, and the patients return with alacrity to their daily avocations. But men without a spleen are disposed to contract malarial infection just as before, nor does the course of the disease present any manifest clinical differences. Tricomi, who has performed seventeen splenectomies, reports that in three cases, after the lapse of a few months, the malarial attacks returned. The spleen, therefore, is not necessary to the development of the parasites producing malarial infection.

For traumatic *rupture of the spleen* laparotomy has been performed, even with extirpation of the organ; the same should be done in cases of spontaneous rupture, if promptly diagnosed and if collapse have not occurred.

Complications.

In the treatment of the complications (pneumonia, dysentery, sunstroke, etc.) we must also, of course, treat the malarial infection. When pneumonia occurs during the course of malaria, besides giving the remedies appropriate to this affection, we must give quinine, regu-

lating the doses according to the condition of the blood with reference to the parasites. It is well to bear in mind that quinine will suffice for the cure of the dysenteric symptoms which sometimes occur in malaria, but that the true dysentery which may be found as a complication of the malarial infection, and which often continues after the fever has been overcome, must be treated by measures appropriate to itself (calomel, hot enemata, opium, etc.). For the insolation complicating malaria, we should give tepid baths and cardiac stimulants, at the same time administering quinine by hypodermic injections.

MALARIAL HÆMOGLOBINURIA.

Synonyms.—Febbre emoglobinurica; fièvre bilieuse mélanurique, hématurique, or hémoglobinurique; blackwater fever; Schwarzwasserfieber.

Definition.—The hæmoglobinuric attack is a syndrome which is encountered not rarely, especially in hot climates, in the course of a malarial infection. The chief symptom of the attack is the emission of urine containing albumin and hæmoglobin in greater or lesser quantity.

All or nearly all authors place hæmoglobinuria among the pernicious fevers. We maintain, however, that a special place should be reserved for hæmoglobinuria among the clinical forms of the malarial infection. The pernicious fevers are grave estivoautumnal malarial infections, the principal cause of which is to be found in the deterioration of the blood through the action of the very great number of parasites, the presence of which is easily demonstrable in the majority of cases in which an examination of the blood is made; the relation between the intensity of the infection and the gravity of the symptoms is usually quite evident. Hæmoglobinuria, on the other hand, is a phenomenon which may be manifested during the course of an active infection as well as when one has spent its force; it is intimately related to malaria, but there is no direct causal relation between this phenomenon and the malarial parasites, such as there is, for example, between the coma of a pernicious attack and the parasitic invasion of the capillary vessel of the brain. Nor do we believe that we can class together, as some have done, hemorrhagic malarial infections in general and hæmoglobinuria. Indeed, cutaneous or mucous-membrane hemorrhage (nasal, intestinal, and the like) constitutes a symptom which may accompany grave malarial infections, while hæmoglobinuria may develop in malarial subjects under the most varied conditions, as will appear in the course of our study. We may add also that, while the pathogenesis of the pernicious

attacks has been in great measure cleared up by the recent parasitological investigations, the same cannot be said of hæmoglobinuria, the pathogenesis of which is not only still obscure, but is also certainly more complex. These points of difference possess great practical importance also as regards treatment. While all forms of the pernicious attack have their sovereign remedy in the salts of quinine, few forms of hæmoglobinuria are cured by quinine, and many indeed are actually provoked by this drug.

History.

The first reports of a "fever with black urine" we owe to French naval surgeons, who described it on the west coast of Africa, especially in Senegal, and in the French colonies in America—Guiana and the West India Islands. In the monographs of Dutrouleau, Bérenger-Féraud, Pellarin, and many others we find a most complete clinical picture of the disease. Recently also several surgeons of the German navy have given excellent descriptions of blackwater fever, among them being Steudel in Bagamoyo, F. and A. Plehn in Kamerun, and Schellong in King William's Land, New Guinea.

Hæmoglobinuria appears to have been wholly unknown to the classical writers on malaria. Thus Torti makes no mention of it in his work, although he had had a most extensive clinical experience. This is perhaps due to the fact that malarial hæmoglobinuria is very rare in our climate, and perhaps also to the fact that cinchona bark had not at that time come into general use in the treatment of malaria; the latter supposition seems the more probable when we remember that the relation between the employment of cinchona preparations and many cases of hæmoglobinuria is undeniable.

To Tomaselli, whose observations were made in Sicily, is due the credit of having demonstrated in a series of publications the intimate relation existing between the exhibition of quinine and the hæmoglobinuric attack. His first monograph, entitled "La intossicazione chinica e la infezione malaria; contributo all' esistenza della febbre ittero-ematurica per la chinina," was presented to the Accademia Giornia of Catania in March, 1874. After him, various observers in Sicily (Ughetti, Cervello, Galvagno, Moscato, and others) and in Greece (Karamitsas), on the strength of independent observations, came to the same conclusions as Tomaselli. But it is only very recently that certain physicians within the tropics have come to recognize the importance of quinine as regards the occurrence of malarial hæmoglobinuria.

The observations carried on in Rome since the discovery of the

malarial parasites have been made chiefly with a view to determine how these parasites act in connection with hæmoglobinuria. It has thus been established that hæmoglobinuria is encountered only in patients who have had the estivoautumnal parasites; that some attacks occur while the malarial infection is still active, and others after the acute infection has spent its force; and finally that quinine is not responsible in all cases for the hæmoglobinuric attack.

As regards the pathological anatomy and the pathogenesis of the affection, very few researches have been made. The most interesting observations concerning the part played by quinine in the pathogenesis of hæmoglobinuria we owe to Murri.

The most important contributions to this subject have been made by Dutrouleau, Bérenger-Féraud, Pellarin, Tomaselli, E. Steudel, E. Rossoni, A. Plehn, F. Plehn, Bastianelli and Bignami, Baccelli, Murri, and A. Berthier. The titles of the works of these authors will be found in the bibliography at the end of this article.

Geographical Distribution.

Icterohæmoglobinuric fever has been observed in all countries where grave malaria prevails, whether tropical malaria or the summer-autumn infection of temperate climates. It is encountered with greatest frequency in the tropics, and especially in certain regions in the tropics. The places where it has been described as occurring most frequently and in most severe form are the west and east coast of Africa, Madagascar, the Congo, the region of the Niger, Sierra Leone, and the Gold Coast. In Algeria Laveran did not see a single case.

O. Dempwolf makes the statement that the only pernicious form of malaria observed by him in New Guinea was the "Schwarzwasserfieber."

In America it has been encountered in French Guiana, Venezuela, Cuba, and elsewhere, but it is apparently unknown in Brazil.

Blackwater fever is found in the Malay archipelago, in the southern part of China, in Assam, and in some parts of India. It is strange that the clasical writers on the diseases prevalent in India make no mention of this affection, a fact which Manson seeks to explain on the hypothesis that they had confused this with other morbid forms or else that it is a disease of recent introduction. It is remarked that certain English physicians in the African colonies also regard hæmoglobinuria as a form of malaria of recent introduction.

In Southern Europe it has been observed with certain degree of frequency (by Karamitsas and others) in Greece, where it has become

epidemic among the laborers employed in cutting a canal through the Isthmus of Corinth, in Sicily (Tomaselli, Ughetti, and others), and in Sardinia (Vincenzi). In the Roman Campagna it is rare in comparison with the other grave forms of malaria. In Central Italy, where malaria is less intense, cases have been seen by Murri and Grocco.

It is important to note the comparative rarity of hæmoglobinuria in the Roman Campagna, although the same form of malaria prevails there in the summer and autumn as prevails in Sicily, Sardinia, and the tropical countries where blackwater fever is of frequent occurrence. It does not appear from the accounts which we have of malaria in the tropics that the grave forms of infection—the pernicious comatose, for example—are more frequent there than in the Campagna, although hæmoglobinuria prevails to such an extent. In certain regions, indeed, blackwater fever appears to be the only grave malarial manifestation. From these facts we infer that this affection is in relation with a special form of malaria, the estivoautumnal, but not with the special intensity with which this form of infection prevails in any given locality. In speaking of the classification of malarial fevers we have not denied that the estivoautumnal species of parasite may present certain varieties which prevail in certain places nearly to the exclusion of other forms, and we have even alleged various facts in support of this opinion. And so we would not deny that a special variety of this species may predominate in tropical regions, differing from the form occurring in our grave malarial infections, not by any fundamental characters of the parasite or by the clinical forms of the disease, but by the property of inducing more frequently in the human organism certain changes upon the occurrence of which hæmoglobinuria depends.

Etiology.

As is well known, hæmoglobinuria is a phenomenon which has been observed in the course of various infectious diseases, such, for example, as scarlet fever, acute articular rheumatism, pneumonia, chronic suppurative conditions, etc. But we must admit the existence of more intimate relations between hæmoglobinuria and malarial infection than between this syndrome and the other infectious diseases. In proof of this is the frequency of hæmoglobinuria in the subjects of malaria, especially in certain climates, and also the great variety of conditions under which the phenomenon is manifested in these malarial subjects.

Such a variety of causal conditions renders the study of malarial hæmoglobinuria one of great difficulty. In fact, recent researches

have demonstrated that there may be cases of hæmoglobinuria with parasites present in the blood, and other cases without parasites in persons who have recently had a malarial attack; and further, there are cases of blackwater fever which are cured by quinine, and still others (in malarial subjects) which occur with such constancy after the exhibition of quinine that we cannot entertain any doubt of the causal relationship.

But in all these cases, although occurring under the most varied conditions, the one fact which can be affirmed with certainty is the etiological importance of the malarial infection. This etiological importance is easily demonstrable in the cases of intermittent hæmoglobinuria cured by quinine. And it is also evident in those cases which are, on the contrary, provoked by quinine, for as yet cases of hæmoglobinuria due to the action of quinine have been observed only in the subjects of malaria either actually present or recently past, and also in those persons only who have long suffered from this infection. Finally in those forms of blackwater fever, whether mild or fatal, in which the malarial infection has run its course, we are unable, for epidemiological reasons, to question the etiological influence exercised by the past infection.

From all that, therefore, we may conclude that malaria can produce certain modifications in the organism, in a special organism, which under various conditions may eventuate in hæmoglobinuria. Undoubtedly the complex of etiological factors, given which hæmoglobinuria occurs as a necessary consequence, escapes our notice for the most part; but we may assert that in such complex malaria holds the first place.

Given the imperfection of our present knowledge, we are obliged to divide the hæmoglobinurias occurring in malarial subjects, in a purely nosographical sense, into several groups, and as the criterion for such distinction we take in the first place the action of quinine, and in the second place the presence or absence of parasites in the blood.

Thus in the first group we place all those cases of blackwater fever which occur without our being able to invoke the toxic action of quinine, and this group we further subdivide into two, according as the malarial infection is still active (parasites being present in the blood) or has run its course. And since in these cases the etiological influence of malaria is alone in evidence we shall call them a potiori *malarial hæmoglobinuria*.

In a second group we place all those cases in which the influence of quinine as an exciting agent of the attack, as the occasion of the hæmoglobinuria, is manifest. And for the same reason given above,

since the toxic action of the quinine is chiefly in evidence, we shall speak here of *quinine hæmoglobinuria*.

This distinction ought to be retained, in part at least and provisionally. It may happen indeed that in the same individual, in the various relapses of malarial fever, we may observe not only malarial attacks (with parasites in the blood) without hæmoglobinuria and attacks with hæmoglobinuria, but also attacks of hæmoglobinuria after the administration of quinine and the administration of quinine without consecutive hæmoglobinuria. This forces us to assume the existence of some other causal factor as yet unknown, the cooperation of which is indispensable in order that the phenomenon may be produced; and this being taken away, neither the malaria *per se* nor the quinine *per se* is capable of exciting an attack of hæmoglobinuria.

But the difficulty of classifying some cases does not prove that the classification above given is not opportune. We hold that an analytical exposition of the facts is indispensable now for the reason that it may lead in the future to a better comprehension of the facts themselves.

Among the occasional causes of an attack of hæmoglobinuria, in addition to the action of quinine, some authors allege physical injuries, mental excitement, catching cold, etc.

Symptoms.

The attack of hæmoglobinuria, after certain prodromes which are in no sense characteristic, begins generally with a severe and prolonged chill and violent vomiting. Shortly afterwards the patient voids urine containing albumin and hæmoglobin; only in exceptional cases the hæmoglobinuria precedes the chill (Berthier). Then in the course of a few hours the patient becomes jaundiced, the greater or lesser intensity of the icterus depending upon and indicating the gravity of the attack.

The vomiting of a greenish liquid may continue throughout the course of the attack, being so incessant as to prevent the ingestion of even the smallest quantities of water; and with the vomiting is usually associated a bilious diarrhœa. A feeling of thoracic oppression, which may be excessive, and not rarely also pain in the epigastrium, hypochondria, and lumbar regions, together with the obstinate and violent vomiting, are the symptoms causing the greatest distress to the patient. From the beginning micturition is frequent.

The temperature rises rapidly to 40° C. (104° F.) and over, and it is only in exceptional cases that it does not rise above 38° C. (100.8° F.). It continues with irregular remissions up to the end of the attack.

The attack is often of short duration, sometimes less than twenty-four hours, but more frequently it lasts for from four to six days. The prolonged attacks are usually grave, but exceptionally we may see hæmoglobinuria of protracted course which is of slight intensity and is recovered from spontaneously, as in one of the cases reported below, in which the affection lasted for about two weeks without the occurrence of any grave symptoms.

In grave cases we find pronounced psychical depression, severe headache, and perhaps profound somnolence, or instead agitation and delirium.

As regards the abdominal symptoms, in addition to the pain and tenderness on pressure in the epigastrium and in the hypochondriac and lumbar regions, we often note marked meteorism. When this is not present physical examination reveals a very appreciable increase in volume of the liver and spleen.

The termination, even in grave cases, may be in recovery, and even a spontaneous cure is not rare. Sometimes following the hæmoglobinuria there is fever without urinary changes which lasts several days and may be accompanied with typhoid symptoms, or at other times the patient is ill and presents symptoms of nephritis. Death may occur during the attack with symptoms of cardiac paralysis. In other cases the patient falls into a sopor and coma, the urine decreases progressively in amount, and death follows a period of anuria. Thrombosis of the heart is, according to F. Plehn, the cause of death in the greater number of cases.

The fundamental symptoms of the attack are the hæmoglobinuria and the icterus. The latter may be absent in mild cases, as we see in the first of the illustrative cases recorded below. But with this exception it is one of the most constant symptoms of malarial hæmoglobinuria, although, as we know, it is often absent in the other forms of the affection. In severe cases the patient may within a few hours acquire a lemon-yellow color, and this later becomes a brownish-yellow (F. Plehn). But it goes away as rapidly as it came on, and we may see a most intense icterus disappear completely within three or four days after the subsidence of the hæmoglobinuria.

Examination of the urine furnishes us with the most characteristic data. In mild cases the urine is not diminished in amount, and it may even be increased, and micturition is performed normally. In the more severe cases, however, the patient passes with tenesmus and much pain a small quantity of urine (50 to 100 c.c., 1½ to 3 oz., in the twenty-four hours) or even only a few drops. There are also cases in which, a few hours after the onset of the attack, the renal function ceases entirely. The color of the urine is from claret-red to port-wine-

red and reddish-black. The reaction may be weakly acid (diminished acidity), neutral, or even alkaline. The amount of albumin oscillates between one-half and two per thousand or even more. Most authors (Karamitsas, Fisch, Schellong, A. Plehn, F. Plehn) have failed to demonstrate the presence of bile pigments in the urine (Gmelin-Rosenbach test) in cases of hæmoglobinuria, even when the jaundice was intense. F. Plehn obtained positive results in only two cases of intense icterus. On spectroscopic examination we obtain the lines of methæmoglobin or of oxyhæmoglobin. In all the cases observed in Rome the spectrum of methæmoglobin was obtained, except in one rather mild case in which we found the lines of oxyhæmoglobin.

Microscopical examination of the sediment, which is usually quite abundant, gives important results. Hyaline and granular casts are found, often stained with hæmoglobin and containing granules of this substance; epithelial casts stained with hæmoglobin, and also some of normal color; renal epithelium, swollen and disintegrated, and also leucocytes. But the greater part of the deposit consists of unformed masses of hæmoglobin granules of a reddish-yellow color. Not rarely we find also a few red blood corpuscles, and in some cases red corpuscles are found in the sediment in quite appreciable numbers during the entire course of the attack; most of them are altered in appearance. Not uncommonly we see the urine resume its normal appearance, containing only a little albumin, a few hours after the beginning of the attack. In other cases there is established an albuminuria which lasts several days or even weeks.

Examination of the blood gives various results according to the presence or absence of parasites. From this point of view we have to distinguish: (1) Cases in which we find in the blood estivoautumnal parasites in the febrifacient stage; (2) cases in which we find only crescents or pigmented leucocytes, pointing to a recent infection which has run its course; (3) cases in which no parasites at all are found in the blood. In every case, however, in which parasites have been found they have been of the estivoautumnal variety. There is reason to believe that the cases belonging to the third category are very numerous; but in order to determine the frequency with which the parasites are found in the hæmoglobinurias of the tropics much more extensive researches are necessary than we at present possess.

From the morphological point of view, the blood of those suffering from hæmoglobinuria, the patients being already anæmic in consequence of the malaria, presents in most cases the appearance and characters of anæmia, namely, the presence of nucleated red corpuscles and even of megaloblasts.

The diminution in the number of red corpuscles and in the amount

of hæmoglobin following an attack is ordinarily very marked; in grave cases it is not rare to find the red corpuscles reduced in number to about one million, and the amount of hæmoglobin diminishes in a parallel line. In one fatal case F. Plehn found the hæmoglobin reduced to sixteen per cent., and Steudel found it in even less amount. On the other hand, at the beginning and during the course of the attack no notable diminution in the number of the red corpuscles can be discovered; the explanation of this is that the diarrhœa, the profuse sweating, and the obstinate vomiting, together with the absolute or nearly absolute impossibility of introducing any liquids into the system, cause a thickening of the blood.

As regards the state of the white corpuscles, Bastianelli and Bignami have found in various cases a leucocytosis (increase in the number of leucocytes with polymorphous nuclei) which can be recognized from the beginning of the attack. But this is not constant. Bignami found no leucocytosis in one case of hæmoglobinuria with protracted course; and F. Plehn noted in his cases a rather small proportion of leucocytes, but we do not know whether his counts were made after or during the attack. Bastianelli and Bignami have noted also in some cases a marked increase in the number of blood plaques, which were sometimes of larger size than normal.

In regard to the morphological alterations of the red corpuscles, we have sometimes found none, but often we see many macrocytes, and much less commonly microcytes and poikilocytes; indeed, in the cases studied at Rome by Bastianelli and Bignami poikilocytes were never seen. It is also rare to find shadow cells. As a general rule we find no evident alterations in fixed preparations stained after Ehrlich's method.

Examination of the serum from the blood taken during a paroxysm has given various results at the hands of different observers. F. Plehn found it of a more or less reddish color, except in mild cases in which the color was normal. Berthier in two cases found the serum of normal color, but spectroscopic examination showed the presence of hæmoglobin. In one case of protracted course, but of moderate intensity, Bignami found the serum normal in color.

Finally, we may add that in two cases Bignami found a marked tendency to agglutination in the red corpuscles.

The *course* of an attack of hæmoglobinuria may vary in duration and in gravity, as we shall see when we come to describe the clinical forms of the disease. We have already spoken of the duration. As to the gravity we may distinguish, following some authors (Bérenger-Féraud, for example), a light form, a form of medium intensity, and a grave form. The forms observed at Rome of intermittent hæmoglo-

binuria with repeated attacks of short duration were in general not grave. The cases with protracted course are as a rule of considerable gravity; but to this rule there are exceptions.

A fatal result in the grave attacks is frequent. The mortality rate varies according to different writers within rather wide limits, from between nine and ten per cent. up to fifty per cent. (Mannaberg). It may be that the gravity varies in different places. Probably also treatment has much influence, for it can readily be understood that treatment of the quinine form of hæmoglobinuria with repeated doses of quinine, which some physicians who refuse to admit the toxic action of the drug in these cases give at the present day, cannot be other than harmful.

A spontaneous cure is, however, far from rare even in prolonged and grave cases, as has repeatedly been observed in the tropics (A. and F. Plehn). A. Plehn has noted that in those cases in which parasites were found in the blood, these without exception disappeared during the attack; and he interprets this fact by assuming that the red corpuscles which are invaded by the parasites (estivoautumnal) are rapidly destroyed (dissolved). But as nearly all Plehn's patients had taken quinine before the attack we are justified in believing that the disappearance of the parasites was due to the action of this drug. In such a case we could not speak of a spontaneous cure, in the strict sense, of the malarial infection, but only of that of the hæmoglobinuric attack. In one case observed by Bignami there was spontaneous cure of the hæmoglobinuria, although the parasites persisted in the blood and disappeared only when quinine was given.

Convalescence is naturally long when the attack leaves the patient in a seriously anæmic condition, and when especially, owing to the state of the stomach and intestine, normal nutrition cannot be accomplished; and this is frequently the case.

Diagnosis.

The diagnosis is arrived at by taking count of the fundamental symptoms of fever, icterus, and hæmoglobinuria. For absolute security in the diagnosis it is necessary either to be certain of the previous malarial infection or to be able to demonstrate the presence of the parasites in the blood. In regions in which yellow fever and malaria coexist a differential diagnosis is indispensable, but it is not usually difficult for experienced physicians to recognize the two diseases. For a discussion of the points of differential diagnosis the reader is referred to the article on yellow fever in this work, to Sternberg's work on Malaria (New York, 1884), or in general to any treatise on tropical diseases.

Prognosis.

The prognosis of an attack of hæmoglobinuria varies according to the intensity with which it begins. All authors are agreed in saying that we must take into consideration especially the condition of the heart, the renal functions, and the nervous symptoms (delirium, sopor, coma, and convulsions).

The cardiac weakness may be extreme during the attack, calling for a most energetic symptomatic treatment; but we see, nevertheless, cases with protracted course in which, notwithstanding the threatening signs of cardiac failure, recovery ensues.

Oliguria and anuria aggravate most seriously the prognosis when they are protracted beyond twenty-four hours. Some authors, however (F. Plehn, for example), have seen a few cases in which the renal function was reëstablished after anuria lasting several days, and the attack ended in recovery.

Methæmoglobinuric nephritis is generally recovered from in a short time.

A not rare cause of death after the attack consists, according to F. Plehn, is thrombosis of the heart and great vessels. In some cases death may occur from five to eight days after the attack, with symptoms of cardiac failure or with embolism following some sudden movement of the patient.

In our climate it is not rare to see patients who have had but one attack of hæmoglobinuria, but in the tropics the attacks frequently recur—according to F. Plehn, in twenty per cent. of the cases.

Clinical Varieties.

Hæmoglobinuria During the Malarial Attack.—This variety of hæmoglobinuria, which is marked by the presence of the parasites in the blood, comes under clinical observation in one of two principal forms: (*a*) intermittent hæmoglobinuria, and (*b*) hæmoglobinuria with protracted course.

(*a*) *Intermittent hæmoglobinuria* is the form in which, by reason of the presence of parasites in the blood, the beneficent action of quinine, and the intermittence of the fever and of the symptoms which accompany it, the etiological influence of malaria is the most obvious. It occurs as febrile paroxysms accompanied by hæmoglobinuria, following each other at short and irregular intervals, during which not only the fever, but also the hæmoglobinuria ceases entirely. It is a very rare form, at least in our climate. Marchiafava saw one case in the person of a traveller returning from the Congo, in whom he found the

estivoautumnal parasites; the patient rapidly recovered after the administration of quinine in ordinary doses. Another case has been reported by Rossoni; the patient had three very distinct attacks of hæmoglobinuria, after which quinine was administered and effected a cure; after about a month and a half there was a relapse of the fever in grave form, but without a return of the hæmoglobinuria. We shall report this case in full among the illustrative cases below.

(*b*) *Hæmoglobinuria with protracted course* is less rare than the preceding; the affection may last several days—four, five, six, or even more. All the symptoms of the attack occur in their typical form.

The results of the examination to determine the manner of action of the parasites in these cases vary considerably.

1. The parasites may be found only at the beginning of the attack, disappearing later with considerable rapidity. This was noted in many observations by A. Plehn, in which the rapid disappearance of the parasites might be attributed to the action of the quinine taken usually before the beginning of the hæmoglobinuric paroxysm. Baccelli saw the same thing happen in one case, of which the following is the history:

P. Vergari, 20 years old, had had fever in June, 1891, followed by many relapses. The last attacks occurred on November 16th and 17th. On November 21st he was seized with fever accompanied by chills and vomiting and later by diarrhœa; he passed urine of a blood color. Upon entering the hospital he received an injection of 2 gm. of quinine. The following day the urine was black. On the same day a few endoglobular amœbæ and pigmented leucocytes were found in the blood. The hæmoglobinuria lasted through November 23d, 24th, and 25th, and during these days the examination of the blood was negative as regards parasites. On each of these days quinine was given by intravenous injection. The attack terminated after November 25th. The next relapse of the malarial affection was not accompanied by a relapse of the hæmoglobinuria.

2. In other cases the parasites continue their development in the blood during the hæmoglobinuric paroxysm. This was noted in the following observation reported by Bastianelli and Bignami:

The patient was a robust individual who had suffered for a long time from malaria, and who had in years gone by had attacks of hæmoglobinuria. He entered hospital on July 22d, 1892, having a high fever and passing black urine, the hæmoglobinuria having, he said, already lasted two days. In the blood were found parasites with central pigment, fission forms, and young plasmodia without pigment in any notable amount. Injections of quinine were given in the evening. On the morning of July 23d the fever still continued and also the hæmoglobinuria. In the blood were found plasmodia in small numbers without pigment and others with pigment granules. The

fever subsided about noon, but the hæmoglobinuria continued until night. In the evening plasmodia were still found in the blood. On July 24th the patient was apyretic and the hæmoglobinuria had also ceased. In the blood crescent forms only were found.

This case is important because it shows that the parasites continued their development in the blood during the attack, even up to fission and the beginning of a new generation. Their final disappearance was evidently due to the action of quinine, as in the cases reported by Plehn and Baccelli. In observation four of Rossoni, reported below *in extenso*, the hæmoglobinuria lasted about six days, never ceasing entirely, but being less intense in the intervals between the daily attacks of fever. The estivoautumnal parasite was found in the blood.

3. In other cases, as in one reported by Bignami and quoted at length below, the parasites, unaffected by the quinine, go through their entire life cycle during the protracted attack of hæmoglobinuria, and the latter ceases although the parasites continue to develop.

In order to explain this diversity of action of the parasites it is evident that we must ascertain first of all in any given case whether quinine was administered or not, and if administered whether it was given before or during the attack.

Postmalarial Hæmoglobinuria.—In this form of blackwater fever there are no malarial parasites of the pyrogenic phase in the blood. The hæmoglobinuric paroxysm may declare itself a short time after the disappearance of the parasites from the blood, or, in other words, a short time after an ordinary febrile attack. In other cases the hæmoglobinuria may appear at a period of time more or less remote from the malarial paroxysm. In the first case on examination of the blood we find only pigmented leucocytes or crescent forms, which latter, as we know, when they are found alone, indicate that an attack of estivoautumnal fever has recently occurred. If death occurs we find no parasites in the viscera, but only the signs of a recent malarial infection. These forms, because of their development after an ordinary access of malarial fever, were called by Bastianelli and Bignami postparoxysmal (emoglobinurie postaccessuali). As an instance of this form we will cite observation No. 3 of Bastianelli and Bignami, as follows:

The patient was a cachectic individual who had had many attacks of malarial fever and also had several times passed bloody urine (hæmoglobinuria). During the last relapse, after the fifth day of fever, hæmoglobinuria appeared and lasted a little more than twenty-four hours (the patient had not previously taken quinine). During the attack no parasites of the pyrogenic cycle were found in the blood of the finger, but only a very few crescent forms; neither were any

active forms found in the blood of the spleen. Following the hæmoglobinuria was an attack of fever lasting three days, during which no parasites were seen. All finally subsided spontaneously, no quinine having been administered. Ten days later there was a new febrile access with ordinary tertian parasites, but there was no return of the hæmoglobinuria.

In those cases in which the hæmoglobinuria appears a certain time after the malarial attack has run its course, neither parasites nor pigmented white corpuscles are found in the blood. To this group of cases belongs the description of postmalarial hæmoglobinuria which follows.

The course and the gravity of the attack may vary greatly. Sometimes the paroxysm is short and fairly mild, as in a case seen by Murri. The patient was a woman who had suffered from quinine hæmoglobinuria, the cause of the attack being made most evident every time that quinine was administered. She also had three attacks of spontaneous hæmoglobinuria of moderate intensity, occurring without her having taken quinine, without the presence of parasites in the blood, and without any other occurrence to which the onset of the attacks could with any degree of probability be attributed.

At other times the attack is grave and may even terminate fatally, as in two cases observed by Marchiafava and one by Ferraresi, in all of which a post-mortem examination was made. So far as we can determine from the scanty clinical data furnished by these three cases, the symptoms and course of this form of the disease do not differ from those observed in the other clinical varieties of hæmoglobinuria. The results of the autopsies made by Marchiafava showed that the malarial affection had fully run its course, but that it was recent; he found, indeed, melanosis in the viscera, pigmentation of the endothelium, etc. Clinically there was nothing found which could afford a clew to the exciting cause of the paroxysm.

Quinine Hæmoglobinuria.—In this form of hæmoglobinuria quinine is the exciting cause of the attack. In some cases of malaria it is impossible to doubt the causal relation between the administration of quinine and the hæmoglobinuric paroxysm, the latter following invariably the administration of the drug within a certain period of time, as in the cases reported by Murri, Vincenzi, and others. But in many cases such a positive conclusion is not justified, for hæmoglobinuria sometimes follows and sometimes does not follow the administration of quinine even in repeated doses, as in two cases reported by Berthier. Still more difficult naturally is a correct appreciation of the cause in those cases in which the hæmoglobinuric attack following the administration of quinine has occurred but once

in the history of the patient, and is not repeated when a relapse occurs and the remedy is again administered, as has several times been observed in Rome. For these reasons it is difficult to determine the frequency of quinine hæmoglobinuria as compared with the other forms of the disease which we may call spontaneous. A study of the literature of the subject, however, teaches us that among the various forms of hæmoglobinuria due to malaria this one, in which quinine is the exciting cause, is the most important and occupies the first place. This may be affirmed especially of the disease as it occurs in the tropics. Of fifty-five cases of hæmoglobinuria observed by A. Plehn in Kamerun, in forty-eight the attack followed the administration of quinine. Of forty-three cases studied by F. Plehn in the same region, twenty-four were found to follow with certainty a few hours after the ingestion of quinine, and for the most part at the very period at which the action of the remedy is greatest. All the cases of hæmoglobinuria observed by Tomaselli in Sicily were due to the action of quinine. A critical examination made by Ughetti of the cases observed by Pellarin in Guadeloupe showed that all the cases of "bilious hæmaturic fever" of this author were instances of quinine poisoning in malarial subjects. R. Koch saw none but quinine hæmoglobinuria on the east coast of Africa.

For a discussion of this particular action of quinine the reader is referred to the section on Treatment ("Quinine Poisoning," page 470). Here we shall limit ourselves to the statement that quinine hæmoglobinuria in malarial subjects may occur under two forms: (1) Those cases in which the hæmoglobinuria manifests itself when the quinine is taken to cure an actual attack of malarial fever; (2) those cases in which the malaria has run its course, but there remains for a long time some unknown modification in the organism in consequence of which the quinine causes hæmoglobinuria.

The cases described by Tomaselli belong almost entirely to the first of these categories. Out of thirteen observations related by this author in two only (Nos. 6 and 7) do we find any cause to suppose that the malarial attack was not present when the quinine produced the hæmoglobinuria. The observations of Plehn also belong to this same group. He says that he has never seen any harmful action of quinine when it was administered in moderate doses in the apyretic intervals when the patient was in a state of well-being, in other words, in that period in which the parasites are absent from the blood. But the hæmoglobinuric paroxysm could be produced by exactly the same doses of quinine when given soon after an attack of malarial fever or when the patient felt ill and took the quinine, fearing an approaching relapse.

In some cases the hæmoglobinuric paroxysm follows with great constancy the administration of quinine taken under the conditions above mentioned; in other cases, although the patient is in apparently identical conditions, the expected effect does not follow. We see this in certain of the cases reported by Plehn and in also some observed in Rome (Bastianelli). In one case, for example, recently observed at the Santo Spirito Hospital, there were three attacks of quinine hæmoglobinuria in three successive relapses of fever. When the fourth attack began the physicians hesitated some time about giving quinine, fearing to expose the patient to a new attack of hæmoglobinuria; but after some days of high fever (estivoautumnal), the patient urgently calling for quinine since he knew by his previous experience that, while it might produce an attack of hæmoglobinuria, it would cut short his fever, the remedy was given and produced the desired effect without exciting another attack of hæmoglobinuria.

To the second group of cases belong one of Murri and several of Vincenzi. But it is to be noted that the two varieties may be observed in the same patient, an attack being produced when the remedy is given to subdue an actual paroxysm of fever, and also when it is given in a prophylactic sense after the febrile attack has disappeared.

The symptomatology of quinine hæmoglobinuria is the same as that of the spontaneous form of the disease.

Pathological Anatomy.

The following description is based chiefly upon the results of observations made in our school of pathological anatomy, and especially upon the findings in five autopsies. In one of these (Case I.) the hæmoglobinuria occurred during the course of an acute malarial infection after the administration of quinine (Bastianelli and Bignami); in a second (Case II.) the hæmoglobinuria began without quinine, but this drug was given during its course (Bignami); in the three other cases (Cases III., IV., and V.) there was a postmalarial spontaneous hæmoglobinuria (Marchiafava), one of the cases being reported in full below.

In these cases the autopsical findings varied according as the malarial infection was recent and intense or had already passed. In the first case the same condition was found in the liver and spleen as is ordinarily found in cases of pernicious malarial disease (softening and melanosis of the spleen, melanotic liver, etc.). In the second case a few malarial parasites (crescents) were found. The last three cases (postmalarial hæmoglobinuria) occupy a special place by reason of the intensity of the icterus and the grave alterations in the

liver and kidneys; in them were found changes due to a preceding malaria, such as the absence of parasites, not very marked black pigmentation of the endothelium of the liver and of the triangular spaces, etc. In addition to these variable changes due directly to the recent, or rather old malarial infection, there were others having a relation to the fatal hæmoglobinuric attack which were found especially in the liver and kidneys.

The *liver* was found increased in volume, congested, and rich in bile; the gall-bladder was distended with inspissated bile, and the intestine also was filled with bile. On microscopical examination in certain cases (I. and II.), no changes were found in the hepatic cells any more intense than those which can be seen in ordinary grave malarial infections; such for example were isolated necrotic cells, or rather little islands of necrosis, etc. In other cases many of the hepatic cells contained yellowish pigment or granules of hæmoglobin, but not always more abundantly than we see frequently in cases of ordinary pernicious fever. The capillaries for the most part contained a large number of leucocytes. Deserving of special mention are the changes found in the last three cases (postmalarial hæmoglobinuria) and particularly in one in which death occurred with very intense jaundice after a period of anuria. In this case what attracted attention particularly at the first glance, especially in fresh frozen specimens, was the distinctness with which the network of pericellular biliary capillaries stood out, owing to their great injection and distention with bile. This biliary injection was most manifest in the centre of the hepatic lobule around the rami of the suprahepatic veins. In the hepatic cells were to be seen grave regressive alterations in the lobules arranged in more or less circumscribed zones, such as atrophy, vacuolar degeneration, in some parts necrosis, in others complete disappearance of the hepatic cells in such a way that in some places only the vascular network with Kupfer's cells remained. Many of the hepatic elements were charged with yellowish granules. These changes, as we have said, affected only small tracts of the lobules. On the other hand there were found near these tracts hepatic elements of perfectly normal appearance in the various phases of karyokinesis. The barrel and monaster shapes prevailed, but there were also other figures in which the cellular division was already advanced, as for example the dispirema. The number of karyokineses was very much greater than that found in cases of pernicious fever by Guarnieri and Bignami, in which in general the karyokineses were very few, and even in many instances could not be found at all.

The *kidneys* were either of normal volume or slightly increased in

size and congested. In our observations, with one exception, there were no evidences of interstitial hemorrhages. On macroscopical examination there were seen brownish-red or black striæ, especially in the pyramids. In the more grave cases the kidneys were found enlarged, flaccid, œdematous, icteric, with a blackish discoloration of the pyramids and medullary rays.

On microscopical examination marked alterations were seen in the straight tubules. The glomeruli, except for the melanosis in those cases in which the malarial infection was actually present or recent, presented no notable changes. Occasionally we found desquamated epithelium and within the capsule a granular mass with colorless hyaline spheres. But the fact is worthy of mention that we did not see any imbibition of hæmoglobin by the cellular elements, nor were there any granules of hæmoglobin within Bowman's capsule. In many of the convoluted tubules there were evident epithelial changes, such as turbid swelling, a disintegration and breaking down of that part of the protoplasm which lies nearest the lumen of the tubule, fatty degeneration, and in some cases a true coagulation necrosis of the epithelia. In the second case the greater part of the tubules so markedly altered contained within their lumen only a finely granular amorphous detritus, without any hæmoglobin, while in those tubules which contained hæmoglobin cylinders the epithelium was generally preserved, being flattened against the walls of the tubules, having a nucleus readily stained with hæmotoxylin, and protoplasm sometimes containing hæmoglobin. Other of the epithelial cells, however, were loaded with granules of a light yellow color (hæmoglobin) like those constituting the cylinders of hæmoglobin contained within the tubules. Some of the cells were infiltrated with hæmoglobin. In the three other cases the same alterations were found, though more marked in degree, and there were also cylinders loaded with bile pigments.

The varied aspect of the renal casts, noted especially in the last three cases of the series, is worthy of note. The different kinds were as follows: casts formed of granules or blocks of hæmoglobin and others of filaments stained with hæmoglobin; casts like the preceding in which, mixed in with the mass of hæmoglobin, were small uninuclear cellular elements having but little protoplasm stained with hæmoglobin; casts formed of little spherules, the size of a red blood cell, which took on an indistinct greenish-yellow tint (preparations stained with hæmatoxylin and eosin); casts formed in part of hæmoglobin detritus, in part of red blood corpuscles which were altered but still recognizable; within some tubules were found accumulations of leucocytes, for the most part uninuclear (lymphatic

corpuscles), sometimes with protoplasm stained with hæmoglobin or bile pigments; finally casts were seen which consisted of epithelial cells with readily stainable nucleus and protoplasm strongly impregnated with hæmoglobin.

In Henle's loops were found the various forms of cylinders described above; the investing epithelium of the loops was generally found well preserved. In the tubules in the medulla and pyramids the casts were more numerous than in the cortex, whence the macroscopical appearance of the pyramids. The lining epithelium in general presented no marked alterations and it was not infiltrated, as was that of the convoluted tubules, with pigmentary substances. But there were also straight tubules containing accumulations of hæmoglobin granules in which the epithelium was disintegrated and mixed in the lumen of the tubule with the masses of hæmoglobin. Many of these tubules were dilated and formed little pouches in the part occupied by the detritus above mentioned. Around some of these pigmented cylinders, especially in the last three cases—those in which the bile pigments prevailed, there was sometimes seen an evident proliferation of the epithelium of the tubules, giving an appearance as of rings and crescents resting upon these central pigment masses. This was seen most clearly in the straight tubules, but occasionally also in the convoluted tubules. Finally among the epithelia of the convoluted tubules and of the loops of Henle a very few elements could be found here and there in process of mitosis.

In the interstitial substance, except for intense hyperæmia, no alteration is found as a rule. In one case only we found here and there little accumulations of epithelioid cells with which leucocytes were mixed, which formed clearly circumscribed small tubercular nodules; these seemed to be found, however, only among the tubules of the pyramids. (For these histological lesions of the kidneys, see Plate XI.)

The *spleen* in some cases presented the same macroscopical and microscopical appearances that are seen in general in acute and chronic malaria. Nucleated red blood corpuscles were often found there. We never saw a greater number of globuliferous cells or those containing yellowish pigment than can be found in ordinary cases of grave malarial infection.

The *bone marrow* showed essentially the same lesions as are seen in ordinary cases of malaria.

The *heart* usually had its ventricles dilated. Sometimes it contained a little fluid blood, at other times clotted masses. Not rarely there were subpericardial hemorrhages.

Pathogenesis.

The etiological conditions under which the various forms of hæmoglobinuria occur are multiple and complex. Of first importance we must regard the variety of malaria, for in fact hæmoglobinuria is found only in individuals who have or have had an estivo-autumnal infection. In the second place we must take account of the alterations in the organism produced by a more or less extended series of malarial fevers. The first condition we must regard as necessary, but as not in itself all sufficient; necessary because up to the present we know certainly of no cases of malarial hæmoglobinuria connected with the development of any other species of parasite, and because, in the cases recorded, when the parasites were suppressed by means of quinine the hæmoglobinuria was also caused to disappear; not, however, in itself all sufficient, because in very many cases when a relapse occurs the hæmoglobinuria does not regularly return.

The etiological importance of the estivoautumnal parasite must be conceded for all the clinical forms of hæmoglobinuria which we have described. There can of course be no doubt of this in the case of those forms of hæmoglobinuria which develop while the malarial attack is actually present. But also in many cases in which the hæmoglobinuria comes on after the malarial attack has run its course, or in consequence of the administration of quinine, there is good reason to regard as indispensable the condition that the infection which has gone before shall have been an estivoautumnal fever. This can be demonstrated in many cases. But the fact that hæmoglobinuria in its various forms prevails especially in tropical regions in which pernicious fevers also prevail, and that it usually attacks an individual only after a long sojourn in the infected district, is a strong argument in support of the contention that the etiological influence of this species of malaria is indispensable.

The second condition may be demonstrated, according to the unanimous testimony of all those who have had experience with the affection, in all cases. We ourselves know of but one case of malarial hæmoglobinuria (observed at the Hospital of San Giovanni) in which the attack, which was cured with quinine, occurred after only a few paroxysms of a primary estival infection. But this observation is exceptional, and because it is exceptional may be looked upon as of doubtful authenticity. We must therefore regard as an indispensable factor in the production of hæmoglobinuria those alterations in certain viscera, bone marrow, liver, spleen which are the invariable consequence of the malarial infection.

But this is not all. Since hæmoglobinuria is encountered rarely in temperate climates and frequently in tropical climates, and especially in certain definite tropical regions, we ought, some authors believe, to admit the action of a climatic factor which, in the present state of our knowledge, we are unable to analyze. It may be assumed that this climatic action is exerted only upon the hæmatopoietic organs and the blood, which action is admitted by many authorities. We may note, however, that importance has come to be given to the influence of climate because of the fact that there are regions of malarial hæmoglobinuria just as there are regions of benign tertian fever. Now we may possibly explain this climatic influence, assumed by some to exist, as nothing more than some peculiar property of the malarial agent which dominates in those regions where hæmoglobinuria prevails. We have stated in a preceding section that the tropical fevers are produced by the same species of parasites as the estivoautumnal fevers of the Roman Campagna. While speaking of this species of malaria, in discussing the classification of the fevers, we stated that it was a single and very widely diffused species, but in all probability including several varieties, distinguished one from the other more by the type, the course, and the gravity of the fevers which they caused than by any morphological peculiarities. In other words, we held that the same species of parasites may present in certain places special varieties distinguishable from each other by the pathogenic action which they exert upon man, and not by any fundamental biological characters. It seems to us now very likely that the malarial affection, in the course of which hæmoglobinuria frequently occurs, represents one of these varieties. We are led to this manner of thinking also by the fact that it is not in all places within or near the tropics in which the tropical fevers properly so called (estivoautumnal fevers) prevail that hæmoglobinuria is equally frequent. For example, it is very rare in Algeria while it is of extremely frequent occurrence on the west coast of Africa, and is rare apparently in India. It is very probable that these differences are due to varieties of the pathogenic agent rather than to the action of a hypothetical climatic factor.

Finally, since under the conditions above mentioned not all malarial subjects suffer from hæmoglobinuria, in our climate very few indeed, we must assume that those who do suffer from it must have reacted to the malaria in a special manner. In what this individual factor consists we are unable to say, although its existence is amply demonstrated. Many authors speak of an idiosyncrasy, and we shall adopt the word, although we intend to indicate thereby nothing more than the existence of an unknown individual factor.

But the importance of this individual factor is demonstrated especially by the differences as regards susceptibility to malarial hæmoglobinuria among the various races. It is known that the different races of negroes are unlike in their behavior as regards malaria; in some places hæmoglobinuria occurs among them, but out of two hundred and seventy-six cases of malarial fever observed by F. Plehn among the aborigines on the Kamerun coast there was not a single case of hæmoglobinuria, while the affection was very common among the white residents of the same region.

As regards the *pathogenesis* very little positive can be said, because of the complexity of the phenomena and the paucity of carefully observed cases. The anatomico-pathological alterations described, and especially those of the liver and spleen, furnish us with certain data which may be utilized in this study. In some cases these alterations are not very intense and resemble closely those observed in experimental hæmoglobinuria, as that induced by distilled water, by glycerin, etc. In other cases, as in the last three of those studied by us, they are very grave and recall those seen in hæmoglobinuria provoked by toluilindiamin (Afanassiev).

The hepatic lesions are evidently in relation with the increased afflux of material destined for the formation of bile, whence the intense polycholia, the injection of the biliary capillaries, etc. The great number of hepatic cells in process of mitosis, found in one case, ought probably to be regarded as secondary to the stimulation by the bile retained in great quantity in the biliary capillaries. We know indeed that even in cases of simple ligature of the ductus choledochus the resulting stagnation of bile within the liver produces regressive cellular changes as well as progressive modifications (mitosis) of the hepatic cells.

The renal alterations vary according to whether there has been an elimination simply of hæmoglobin and albumin (as in our Cases I. and II.) or also of bile pigments (as in Cases III., IV., and V.); the lesions in the latter case are more grave than in the former. It may be stated first of all that the changes in the glomeruli, even when they are visible to the naked eye, are always, apparently at least, of slight importance, and nothing discoverable on histological examination would lead us to suppose that hæmoglobin is eliminated through them. Everything, however, causes us to believe that the hæmoglobin is eliminated by means of the epithelium of the tubules, and especially, if not exclusively, by that of the convoluted tubules. Indeed, in the convoluted tubes we find epithelial cells containing granules of hæmoglobin and also epithelial cells, the protoplasm of which presents the appearance of being impregnated with this sub-

stance; and besides we find the most varied regressive changes from simple cloudy swelling to coagulation necrosis. These last-mentioned changes are probably the consequence of the eliminative work in which the epithelial cells are actively engaged; indeed, even in the convoluted tubules in which the epithelium is most gravely altered we find no evidences whatever that there is a passive infiltration of hæmoglobin. It is most probable that the anuria with which certain cases terminate is due to the extensive alterations in the secreting epithelium, and that it is not owing to the passive blocking up of the tubules with casts of hæmoglobin, as several authors believe.

But the changes above described are important especially as regards the question of the part taken by the kidneys in the elimination of the hæmoglobin. Ponfick's theory is that when the destruction of the red corpuscles occurs in the circulation, the fragments and the shadows of the corpuscles accumulate in the spleen, while the coloring matter is taken up by the liver and transformed into bilirubin; and it is only when these organs are incapable of working over the remains of the corpuscles and transforming the hæmoglobin that the kidneys intervene and eliminate the excess of this substance.

Against this theory Murri has cited many facts and opposed many arguments. He has noted, for example, that hæmoglobinæmia may exist for hours without there being any hæmoglobinuria, which leads us to believe that the kidneys do not perform the merely passive function of filtration. Arguing also from the fact that an accurate examination of the urine shows that a renal modification precedes the occurrence of hæmoglobinuria, Murri comes to the conclusion that the kidneys do not allow the hæmoglobin to pass until they have begun to suffer in the integrity of their functions.

Our own histological observations have led us to a theory which is quite similar to this. Indeed, many hold to the opinion of Ludwig that the function of the glomeruli consists in a pure filtration, but on the other hand no one doubts that the epithelia of the convoluted tubules have an active function in regard to the materials that are brought to them by the capillary current. Then since the hæmoglobin is removed, as we hold, by the epithelium we can exclude the hypothesis that it is eliminated by simple filtration, and we are forced to the conclusion that the epithelial cells become actively charged with this material in order later to excrete it.

The results of a histological examination of the kidneys also lead us to believe that the hæmoglobin is eliminated in part in solution, in part under the form of granules or fragments which may be found in such abundance in the urine as to constitute the greater part of the sediment. This leads to the further belief that the destruction of

the red blood corpuscles during the febrile paroxysm takes place through fragmentation (erythrorrhexis), especially in those viscera in which we find deposited the greatest quantity of these same fragments and their derivatives, that is to say the liver and kidneys. We cannot conceive of this fragmentation as anything else than the consequence of a preceding corpuscular death. And since the fragments tend naturally to be arrested in the organs in which they originate, we can readily understand the absence of altered red corpuscles in the peripheral circulation, as was noted by us, or their extreme rarity, as reported by others. These fragments would be in part eliminated as such, in part their hæmoglobin would be dissolved out; and so to erythrorrhexis would be added erythrolysis. And since this last phase of the process would take place in the circulation in the same viscera in which the fragments were deposited, which viscera hold and eliminate the broken-up red corpuscles, we find an explanation of the contradictory results obtained by various authors who have made an examination of the serum during the attack, some of whom found dissolved hæmoglobin, while others found none.

This mode of conception of the hæmatic theory avoids certain objections which have been raised against it. Just as in the other forms of paroxysmal hæmoglobinuria, we find here also those who hold to the hæmatic theory, according to which the dissolving out of the hæmoglobin takes place in the circulating blood, and the partisans of the renal theory. The latter base their belief on the fact that we often fail to find hæmoglobin dissolved in the serum of blood drawn by phlebotomy during the attack of malarial hæmoglobinuria; some, as Berthier, have never found the serum colored. In the second place they rest upon the anatomico-pathological findings in the kidneys, in which we note an intense congestion and often also hemorrhagic infarcts. As to the first argument, we may remark that the fact of not finding colored serum during the attack does not exclude the possibility of a destruction of the red corpuscles in the vessels; indeed, there is nothing which forbids us to believe that such a destruction may take place only within the vessels of certain viscera, which suddenly attract, so to say, and rapidly eliminate the products of such destruction. Furthermore, as we have already noted, this absence of coloring in the serum may be explained in certain cases by recalling the fact that the destruction of the red corpuscles takes place, according to our observations, in two stages, and that fragmentation and agglutination of the red corpuscles precede their dissolution; the corpuscles become agglutinated and fragmentation occurs in the circulation of these very viscera which are charged with the final disposition of the products of this dissolution.

On the other hand, against the hypothesis of the renal origin of the hæmoglobinuria are the changes in the liver demonstrable by both clinical and anatomical examination. It may also be said that the theory that the hæmoglobinuria is due to congestion and hemorrhagic infarction of the kidneys is one which finds no support in what pathology teaches us of the consequences of such renal lesions; and it is further to be noted that renal hemorrhages are not always found, and indeed they were not found in the cases examined by us.

But what are the factors of this necrosis and dissolution of the red blood corpuscles is very difficult to determine. As we have already said, although we may affirm that all the clinical forms of hæmoglobinuria found in malarial patients are certainly connected with the malarial infection, yet we are ignorant of the intimate relation between this phenomenon and the infection itself.

The forms of malarial hæmoglobinuria in which the parasites are found in the blood and which are curable with quinine, would seem at first sight more readily interpreted than the others. It is known that the estivoautumnal parasites can cause an early death of the red corpuscles and dissolution of hæmoglobin, as is shown by the fact that we sometimes find parasites within decolorized erythrocytes. Now some have assumed that in certain cases this mode of death of the red corpuscles, for some reason unknown to us, prevails over the other modes (crenation, the formation of black pigment, etc.), and that there then occurs a liberation of hæmoglobin in the blood in the quantity that is necessary, according to Ponfick, to produce hæmoglobinuria. But this interpretation cannot stand against criticism. Before all we find as the result of numerous observations that there is no relation whatever between the number of parasites found in the blood and the gravity of the hæmoglobinuria. Nor do we find in this theory any explanation of why in some cases the hæmoglobinuria may cease while the malarial infection still persists. All this leads us to believe, and with reason, that the causal relation between the parasitic invasion and hæmoglobinuria is not such a simple one as the partisans of this hypothesis would have us believe.

And on the other hand when we come to study the forms of hæmoglobinuria which follow the administration of quinine, we cannot even here admit that the quinine acts simply as a hæmolytic poison. The experimental proof that quinine by itself alone dissolves the red corpuscles in those subjects in whom quinine hæmoglobinuria is seen, is completely wanting. Nor, admitting this hæmolytic action of quinine, can we understand why within short periods, under apparently identical conditions, hæmoglobinuria

sometimes follows and sometimes does not follow the administration of quinine. We may conclude therefore that, while there can be no doubt that in some malarial subjects hæmoglobinuria follows, sometimes with great constancy, the administration of quinine, yet the quinine itself does not act by dissolving the red blood corpuscles like a hæmolytic poison, but acts in some altogether special manner.

Murri, who investigated the pathogenesis of quinine hæmoglobinuria in a typical case of the disease, was unable to see that the blood of his patient, mixed with hydrochlorate of quinine dissolved in variable proportions in physiological salt solution, and kept for several days, acted in any other way than the blood of a healthy person so treated. Quinine therefore does not exercise its solvent action directly upon the red corpuscles, nor does it act directly upon the plasma by changing its composition. But Murri adds: "Whoever would assert, however, that this chemical change in the plasma cannot occur during life would go beyond what the experiment gives justification for, since the quinine acting upon the various functions might induce indirectly a chemical change in the plasma in which the red blood corpuscles could no longer maintain their physiological unity." And he concludes that between the quinine and the red corpuscles there must be some intermediate factors which lead indirectly to hæmoglobinuria.

As regards the cases of spontaneous hæmoglobinuria which occur in malarial subjects in whose blood no parasites can be found or who have not been taking quinine, we are unable to say whether any attempt has yet been made to explain them. Neither do we know whether there have been any attempts to explain the fact that the hæmoglobinuria, even in the same patient, may occur under conditions apparently very dissimilar; that is to say, at one time after the administration of quinine, at another time without any quinine having been given, at one time when parasites are present in the blood, at another when none can be found.

This great irregularity might possibly lead us to regard hæmoglobinuria as simply an associated phenomenon or a complication of malaria, as Yersin believed, who discovered in the urine of two patients certain small bacilli which he cultivated and found pathogenic for rabbits and mice; he concluded therefore that hæmoglobinuria has nothing essentially to do with malaria. These observations, however, have not been confirmed. Berthier, for example, collecting the urine with all antiseptic precautions, found no microorganisms in it. But more than this, when we see hæmoglobinuria associated not with malaria in general, but always with a special form of the infection, leaving aside the other considerations referred to above, we cannot

doubt concerning the causal relation between this species of malaria and hæmoglobinuria.

Whoever would undertake to construct a theory regarding the pathogenesis of this morbid syndrome ought to take into account all the etiological conditions and the clinical observations above noted. And in the first place he should explain why hæmoglobinuria, even in those tropical regions where it is most prevalent and in most intense form, does not affect the new arrivals, but only those who have already had many attacks of fever and have become, so to say, acclimated; the affection comports itself, therefore, in a manner entirely different from the usual grave forms of malaria (the pernicious fevers). In the second place he should explain why the affection presents itself under such varied conditions as we have mentioned above; a fact which would lead us to believe that the known etiological conditions (presence or absence of parasites, administration or not of quinine, changes in the viscera due to progressive malaria, etc.) are not sufficient, each in itself, but on the other hand each one represents one factor in a complex cause to which the phenomenon is due.

In conclusion, the facts above noted lead us to believe that the malarial parasites are not the producers of the hæmolytic substance, since in such case we cannot explain why the hæmoglobinuria may cease while the infection continues. It is true that in other cases the administration of quinine and the destruction of the parasites lead to a cure of the hæmoglobinuria; in that case, however, we do not suppress the cause of the hæmolysis, but the occasion through which the hæmolysis has taken place. In the same way it is not the quinine which is itself the hæmolytic substance or which directly causes its formation. All this forces us to the conclusion that the quinine and the parasites are secondary factors of this phenomenon, and that there remains some essential and fundamental factor which is as yet entirely beyond our ken.

What now can we assume in regard to this fundamental factor? Bignami has proposed an hypothesis which is based upon the results of recent experiments concerning hæmolysis. It is known that the hæmolytic property of the serum of one animal in respect to the red corpuscles of another animal resembles the bacteriolytic properties of the serum of an immune animal in respect to the bacteria against which that animal has been immunized. We know, for example, that an animal which has been immunized against cholera has a serum capable of dissolving the cholera vibrios introduced into the peritoneal cavity. In a similar way, if we treat, as did Bordet, a guinea-pig with repeated injections of the blood of a rabbit, the serum of the

former acquires the property of dissolving in vitro the red corpuscles of a rabbit—a property which the serum of the guinea-pig does not normally possess. Preceding the solvent action occurs an agglutination of the red corpuscles. Heating to 55° C. takes away this hæmolytic property, but the serum thus rendered inactive by heating regains its hæmolytic power upon the addition of a small quantity of normal serum from the guinea-pig or even from the rabbit. This hæmolytic property is a specific one; the serum of the guinea-pig possessing this action does not dissolve the red blood corpuscles of another guinea-pig or of a pigeon, and is but slightly hæmolytic as regards the blood of a rat or of some other animals. Bordet has shown further that, if in the peritoneal cavity of a guinea-pig treated by successive injections of the blood of the rabbit there is injected a certain quantity (2 c.c., for example) of defibrinated rabbit's blood, the corpuscles thus introduced are rapidly destroyed, and after a few minutes the fluid withdrawn from the peritoneal cavity is of a clear red color. When a similar injection is made into the peritoneal cavity of another, untreated guinea-pig the red corpuscles are not altered at all at first, but are finally taken in by the phagocytes.

Ehrlich and Morgenroth have shown that the serum of a goat treated for eight months with subcutaneous injections of the blood of a ram acquires the same properties. The serum of such a goat will rapidly dissolve the blood of a ram in vitro, while the serum of an untreated goat possesses no such action.

To explain the phenomena of hæmolysis, as also those of bacteriolysis, it is necessary to assume, according to Pfeiffer, the existence of two bodies—one resistant and specific, the immunizing body, and a very unstable one (the "additional substance" of Ehrlich). The first would be found in the body of the animal in a stable and inactive form. Under certain conditions, for example when the animal is inoculated with cholera vibrios against which it has been immunized, this substance is transformed into an active and specific substance. The same thing takes place in vitro if we add to the immune serum a small quantity of normal serum. In this serum there is then something (the "additional substance") in very minute quantity which changes the inactive substance into an active form; this same "something" in the body of the animal is secreted by the cells of the animal itself so long as the presence of the active cholera vibrios continues. The bacteriolytic (*mutatis mutandis* the hæmolytic) action of this substance is comparable to that of a ferment and is strictly specific. Thus a given serum is active against a single species of bacteria, or is hæmolytic in presence of only a given species of heterogeneous blood.

Similarly we may believe that in some individuals who have been for a long time exposed to the influence of one malarial species (estivoautumnal or tropical infection), there is gradually effected a modification of the plasma in which is formed a substance capable of dissolving the specifically altered red blood corpuscles (a lysin). This substance is normally not free and active in the plasma, but is freed and becomes active when some other influence intervenes which acts like the "additional substance" (*addiment*, of Ehrlich and Morgenroth). This additional substance may in certain cases be represented by the products of a parasitic invasion, in other and much less frequent cases by quinine; thus the parasites, like the quinine, are but the occasion of the hæmoglobinuric attack, not the whole cause. In other cases (postmalarial spontaneous hæmoglobinuria) the occasion of the attack is unknown to us, unless one would regard as important in this respect the fact, which is not infrequently recorded by writers, that the attack came on after a meal.

But in the same individual who has had hæmoglobinuria during a malarial attack and after the administration of quinine, the malarial affection may continue to relapse and the quinine may continue to be given, and yet there is no return of the hæmoglobinuria. This would lead us to believe that the supposed alteration of the plasma is of the kind that exhausts itself in a longer or shorter period in the greater number of individuals; nor is it difficult to believe this if we compare the process by which this hæmolytic lysin is formed to that by which a bacteriolytic lysin is formed in animals treated in a certain way. This, however, does not exclude the fact that in certain cases the peculiar property of the serum remains permanently, as for example in certain cases of quinine hæmoglobinuria.

Certain facts lead to the supposition that the formation of this hæmolytic ferment takes place in the abdominal viscera, and especially in the liver and kidneys.

The hypothesis put forth by Bignami leads us then to the assumption of (1) an alteration in the plasma which is effected little by little as a consequence of a specific change in the red blood corpuscles, through which a certain number of them come to behave in respect to the organism like the corpuscles in the blood of another species of animal; (2) the formation, in consequence of this change, of a substance in the plasma which is capable under certain conditions of becoming hæmolytic.

As appears immediately, the fundamental condition is the specific alteration in the red corpuscles. We may assume as almost definitely established that there are in the blood of malarial subjects, in addition to the gross changes induced by the presence of the parasites, certain

other intimate modifications not discoverable on microscopical examination. It suffices, for example, to remember that in a relapse a parasitic invasion apparently identical with that of the primary attack may cause a diminution in the number of red blood corpuscles much less marked than the one that took place in the first attack. Now alongside of these useful modifications, which we may regard as the result of an incomplete immunization process, we may admit that there may be other harmful ones through which abnormal red corpuscles are formed. The consequence of this specific alteration in the blood would be the production in the plasma of a capability for dissolving the abnormal red corpuscles, in the same way that the serum of a rabbit treated with successive injections of the blood of a guinea-pig acquires after a certain time the property of dissolving the red blood corpuscles of the guinea-pig.

This hypothesis would explain the fact that attacks of hæmoglobinuria occur only after the malarial infection has lasted a long time; since the assumed alteration in the plasma would require a long time for its production. It would also explain the apparent irregularity and accidental character of the phenomenon, which depend upon the complexity of the conditions which are necessary for its production. It further explains the irregularity in the action of quinine and of the existing parasitic invasion; indeed, in the same individual we may have hæmoglobinuria with parasites in the blood without the intervention of quinine, hæmoglobinuria after the action of quinine, and finally spontaneous hæmoglobinuria without the presence of parasites in the blood and without the previous administration of quinine. All this inclines us to assume, as is affirmed in the hypothesis, that the action of the parasites or of the quinine possesses a secondary importance in comparison with some other unknown factor which, according to the theory, is represented by a newly developed property of the plasma. The malarial parasites and the quinine represent only the agent which liberates the hæmolytic substance, like the "addiment" in the experiments of Pfeiffer and Ehrlich; they represent therefore only the occasion of the attack.

It is probable that certain poisons in the blood which produce hæmoglobinuria only accidentally, like toluilindiamin in dogs, act not directly but indirectly by an analogous process.

Treatment.

The main interest in the various clinical forms of hæmoglobinuria above described is connected with the question of treatment, concerning which there is still much contention. We find, in fact, on one

side authors (Steudel, Küchel, and others) who suggest the treatment of tropical hæmoglobinuria with enormous doses of quinine, while others, like Tomaselli and the brothers Plehn, attribute the fatal issue or the grave course of many hæmoglobinuric attacks to the obstinacy of physicians in the tropics in giving quinine.

According to our observations the conduct of the physician ought to vary in the different cases. When hæmoglobinuria occurs in a malarial subject who has not been taking quinine, and the development of the malarial parasites takes place in the blood in the attack or in a succession of attacks at short intervals, a disappearance of the parasites and the cessation of the paroxysms of hæmoglobinuria may follow the exhibition of quinine, as in the cases of Marchiafava, Rossoni, and others. Naturally in postmalarial hæmoglobinuria when there are no parasites in the blood, there is no reason for giving quinine. The only guide then indicating to the physician whether to give or to withhold quinine ought to be the result of an examination of the blood.

In the numerous cases in which a hæmoglobinuric paroxysm comes on from two to six hours after the administration of quinine it will be well for the physician to refrain from giving the drug in large doses. A careful study of the cases of this sort recorded in the literature shows plainly, we believe, that quinine exercises no useful action upon the hæmoglobinuria even if it does not aggravate it, as it did in many of the cases of Tomaselli and the Plehns. According to these authors the course of hæmoglobinuria treated without quinine is much milder and shorter than that of paroxysms in which this drug is given; and this happens so regularly that we are forced to recognize a specifically injurious action of quinine in these cases. It is unnecessary to add that one is not justified in giving quinine during the attack in those cases in which, by the repetition of the fact, there is no reason to doubt the causal nexus between the quinine and the hæmoglobinuria.

It is difficult to indicate what ought to be the conduct of the physician in those cases in which paroxysms of hæmoglobinuria recur with a certain regularity when quinine is administered in successive relapses. Although in these cases the quinine does not lose its curative action over the malarial infection (Tomaselli), yet the harm which may come to the patient through the hæmoglobinuric paroxysms may be very great. It may be added that the most minute doses of quinine, however administered, and all the preparations that contain quinine may produce in these subjects the same injurious effects. It will be well, therefore, in these cases to limit ourselves to counselling such measures as may favor the gradual attenuation of

the infection until a spontaneous cure is obtained. (See the section on Treatment of Malaria.)

The treatment of the attack itself ought to be symptomatic. Stimulants and inhalations of oxygen are advised when cyanosis and dyspnœa are present, and opium and chloral to combat the agitation; some French writers (Berthier) advise chloroform in these cases. Ergotin has been recommended by some, but rather it would seem in consequence of certain theoretical views regarding the pathogenesis than as the result of sound clinical observation. But it is difficult to determine with exactitude the value of the different measures proposed when we consider that the hæmoglobinuric paroxysm often subsides spontaneously, as we learn from the observations of Kohlstock, Plehn, ourselves, and others.

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MICROORGANISMS.

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Historical Introduction.

The existence of the belief in the capacity of minute organisms to transmit disease may be gathered from the earliest human records. The Roman writer, Varro, concluded that as large insects, such as flies, were capable of doing injury to animals, there must exist still smaller animal forms, some even invisible, equally pernicious in their workings. Paracelsus also would appear to have entertained a belief in living agents as morbific factors, for he speaks of the germs (seminium) of disease. The first important observations upon microscopic forms of life as the cause of disease date from Athanasius Kircher (1671), from whom originated the conception of a *contagium animatum*. "That the air, the water, and the earth vibrate with innumerable insects is so certain that the proof can be brought at once before the eyes. It has been known everywhere in the world that worms develop out of decomposing substances. But it was not until the wonderful discovery of the microscope that it was possible to show that all putrefying substances contain an innumerable brood of minute 'worms' which cannot be made out by the naked eye. This is a fact which I also could not have believed had I not, through many years' repeated observations, convinced myself of the truth of this statement" (Loeffler[1]). The imperfections of the microscopes of Kircher's time did not permit of a more definite description of the minute forms of life which he discovered in decomposing substances. But his observations, imperfect as they were, immediately suggested the far-reaching conclusions that the causes of certain diseases might be sought in some such microscopic living forms.

This train of reasoning is not so difficult to follow when we consider that the prevailing opinions of that time ascribed the origin of disease to some modification, perhaps putrefaction, of a "humor" hidden away in some recess of the body, and that Kircher had convinced himself that whenever putrefaction occurred, the microscope showed the existence of those microscopic forms which he called "worms." The microscope was applied by him to the study of manifold objects—blood, pus, etc.—and he believed that in the pus de-

rived from the lesions of bubonic plague he had discovered similar verminous parasites. Through this observation Kircher believed that he had brought proof of the actual existence of a "contagium animatum."

Advances in our knowledge of microscopic forms of life succeeded rapidly after the greater perfection of the simple microscope (Antony Van Leeuwenhoek, 1695). Leeuwenhoek had already, in 1675, discovered in rain water actively motile animal germs of such extraordinarily small size that the most distinguished microscopist of that time, Robert Hook, was for many years incapable of confirming these observations because of the fact that moulded lenses were employed by him, while those used by Leeuwenhoek were ground and polished.

Leeuwenhoek extended his observations to sea-water, spring-water, vegetable infusions, the intestinal contents of flies, frogs, pigeons, hens, and his own diarrhœic stools, and succeeded in demonstrating the presence of minute living bodies of divers sizes, and distinguishable in respect to form, size, and mode of locomotion. He first accurately described forms which can now easily be identified as bacilli and spirilla.

Great interest was awakened by these discoveries, so that within the next few years material from many sources had been studied with the microscope. Andry (1701) believed that the air, water, vinegar, fermenting wine, old beer, cider, and sour milk were filled with germs; that blood, urine, and the pustules of smallpox contained microorganisms; and that in syphilis no part of the body was free from their presence. Lancisi (1718) ascribed the injurious qualities of marsh air to invisible animals; and Vallisneri (1733), Goiffon, and Lebegue (1721) felt assured that the pest, which prevailed in Toulon and Marseilles in the year 1721, was caused by similar animal forms. Reaction to these far-reaching and often insufficiently founded views was not wanting; but despite the satire and ridicule heaped upon its supporters the idea of the existence of a "contagium animatum" continued to exist. The great Linnæus (1757), knowing nothing of the use of the microscope and regarding with suspicion all observations made with it, himself held that the whole series of minute living forms should be assigned to one class to which he gave the name of "chaos," but nevertheless believed that among these forms the contagium of disease and the causes of putrefaction and fermentation might linger.

Plenciz (1762) showed great acumen in the application of Leeuwenhoek's discoveries. He pointed out that not only was it necessary to assume an increase in and dissemination of the contagium, but also that the "seminium" must for every kind of disease be a special one. Just as one form of plant reproduces a similar form, so there develops

out of the "seminium" of scarlet fever always scarlet fever; out of that of smallpox, always smallpox.

Von Gleichen-Russworm studied the microscopic forms of life contained in various infusions, and was able to discriminate and illustrate twenty-one different types. He described the rod, the vibrio, the coccus, spirillum, and streptococcus, but the scientific value of his observations stands in no proportion to the care and pains with which they were carried out.

The first important attempt to develop a system for microscopic beings that was at all comparable to that applied to the classification of the higher plants was made by Otto Friedrich Müller (1786). Linnæus' "chaos" he included under the name of "infusoria." All characteristics noted, such as form, mobility, habitat, etc., were employed by him in distinguishing the organisms which he had observed.

About the end of the eighteenth century students of microscopic life were concerned less with the description and classification of the various forms then known or just being discovered than in attempting to answer the question of their origin. The proof about this time brought by Redi, Swammerdam, and Leeuwenhoek that insects were not generated *de novo* was not believed to apply to more minute forms of life. The question of the origin of the infusoria continued to occupy the scientific as well as the public mind through the first half of the nineteenth century; and, indeed, it was not until the splendid researches of Pasteur, Tyndall, and others in the third quarter of this century that belief in abiogenesis was finally overthrown.

Among those who held that microorganisms originated from unorganized organic substances were such distinguished men as Needham, Buffon, Wrisberg, Treviranus, and O. F. Müller. The view of spontaneous generation was vigorously opposed by Bonnet (1762) and Spallanzani (1769); and although the evidence adduced, especially by Spallanzani, in order to disprove the conception of generatio æquivoca, was at that time unsuccessful, yet his experiments were conducted in so careful and convincing a manner that even with our increased knowledge, at the present time, they could scarcely be improved upon.

Although Leeuwenhoek had not failed to notice the common occurrence of microorganisms in the higher animals and even in man, it was not until Donné's publication, in 1837, that general interest was awakened in the presence of microorganisms in the secretions, excretions, and pathological products. Donné described "vibrionic bodies" in the pus obtained from syphilitic chancres, and believed that he had proved that these bodies were not present in artificial suppura-

tion produced by the action of vesicants. He regarded the vibrios as the infectious basis of the syphilitic pus which, when inoculated into other parts of the body, set up similar foci of suppuration. Donné, in 1844, retracted his view that the "infusoria," which he had observed in syphilitic pus, were the cause of lues, and admitted that they were probably only accidentally present and did not stand in any etiological relation to syphilis.

The idea of fermentation had from an early epoch been associated with that of disease. The observations of Cagniard, de Latour, and Schwann upon fermenting beer and wine were soon confirmed, and the occurrence of microscopic bodies similar to yeast in the abnormal forms of fermentation, that appeared in various animal secretions and excretions, and especially in the urine of persons suffering from diabetes, was announced. The dissemination of cholera throughout Europe brought the problem clearly before the scientific world, and Boehm (1838) was able to show the existence of yeast-like microorganisms in the dejecta of persons suffering from cholera. Nevertheless, since these bodies were found in the dejecta only in the case of those who had drunk beer, he was inclined to attach but little importance to them.

Bassi's discovery of the cause of muscardine occurred at this time. The same investigator was also able to show that the spores of the fungus were capable of infecting healthy individuals if brought into contact with them directly or through the agency of air currents.

The coincidence of several important investigations in the fourth decennium of the present century gave a new impetus to the study of the relation of parasites to disease and rehabilitated the old idea of a contagium animatum.

Henle (1840–53) sketched with wonderful precision the relation of microorganisms to the infective diseases, and defined the intimate nature, the vital properties, and mode of action of the microorganisms, as well as the dependence of the individual phases and symptoms of the disease in question upon the behavior of the parasites, almost as accurately as has subsequently been done as the result of numerous experiments and direct investigation with optical aids at that time unknown. In his "Handbuch der rationellen Pathologie" Henle writes: "If we trace the miasmatic contagia in their action on the animal organism, we find at once, although with many individual differences, a general and characteristic property which can only be ascribed to living matter, namely, that of multiplying at the cost and by the assimilation of foreign organic material. This conclusion is supported by the course of the great majority of miasmatic contagious diseases. They belong to the group of diseases

which I have termed typical, whose sharply defined stages indicate a development of the cause in accordance with definite laws, such as we find only among living beings.

"What was stated above with regard to the properties of the cause of miasmatic diseases in general holds as good as regards the multiplication of contagia by assimilation. It can, however, be absolutely proved only in the case of the inoculable diseases where we are able to define accurately both the point of entrance and the quantity of material taken up, and the proof becomes the more insufficient the more in any given epidemic the number of the cases produced by miasma exceeds those arising by contagion. That the cause of the disease has multiplied in the region affected by the epidemic is probable whenever the latter spreads gradually from small beginnings and attains large dimensions.

"It is only when its development and reproduction in the diseased body is demonstrated that we are justified in designating the material which occasions epidemic diseases as a *contagium*, and the analogy of the miasmatic contagious diseases with the results of the deposit of parasitic organisms in living bodies previously referred to becomes at once evident. This analogy, as I have indicated above, has led to the discovery of parasites as the cause of many affections formerly termed contagious diseases. There are, however, a number of diseases, in the contagium of which nothing has been found which recalls the forms of known species of animals and plants. Nevertheless, this negative result is not so certain that we can, therefore, absolutely refuse to reckon the contagia among these microscopic parasites. It is not necessary to assume that the organisms which act as contagia are too small for our optical means. But the smallest animals can only be distinguished from the cells, nuclei, and granules which occur in so many tissues and excreta, especially in pus, by their movements, and the smallest plants only in certain stages of their development by the arrangement of their elementary constituents. The granules of which the Botrytis bassiana consists behave exactly like pigment granules or the molecules of pus. It is possible, therefore, that bodies of very various kinds and of great significance may be concealed among the molecules which occur in every microscopical object. It is scarcely necessary to add that these speculations are as yet only hypothetical, but they are not superfluous even in the cases where animal or vegetable parasites have been, or will yet be discovered in the contagium. The question will, however, still remain, whether the parasite is an accidental inhabitant of the contagium and of the diseased body, or whether it is the important active constituent. Much has already been gained by these

views which, though they may only represent a transitional period in our knowledge, will prove a lasting gain. In place of the unintelligible view that the diseased body, or the disease itself, forms the contagious material, we have the opinion that the formation of the contagium is a reproductive process, and that the disease is the result of the reproduction of this extraneous being in the organism and at its expense. From this point of view we must interpret the symptoms of the miasmatic contagious diseases.

"While we must hold that the cause of the miasmatic contagious diseases is a material endowed with independent life, which can reproduce itself after the manner of animals and plants, can increase by assimilation of organic materials, and, growing parasitically on the infected body, can give rise to the symptoms of the special disease; yet the question arises of what the as yet unseen body of this parasite is composed, the result of whose life is so evident and so devastating. It is one of the laws of human phantasy that we must ascribe to the contagium, as soon as we reckon it to be something living, one of the forms which the known organic world presents to our senses; hence in the earlier childish times of research one thought of insects, and when the microscopic animals were discovered, the infusoria could, with still better grounds, be accused of being contagium and miasma. At the present time, since the conclusions that have been arrived at with regard to the fungus of muscardine and similar diseases, it seems more likely that the contagium belongs to the vegetable world, because the extensive distribution, the rapid multiplication, and the tenacity of life of the lower microscopical vegetable beings, as well as the mode of their action on the bodies which they have selected as the seat of their vegetation, present in fact the most remarkable analogies with the infective material of the miasmatic contagious diseases. Muscardine also arises in stagnant marshes, apparently independently, as if it were due to miasma; under the influence of heat and drought it becomes epidemic and contagious. Towards the cessation of the epidemic its contagiousness diminishes, and ultimately becomes lost. Currents of air carry the contagium over long distances, so that the disease appears again in another place, under the aspect of a miasmatic affection. The contagium is an aeriform, and at the same time a fixed body. It retains its power for years in a dry state. An imponderable and incommensurable quantity of it is sufficient to set up the disease, and even to produce devastating epidemics" (Flügge[2]).

The actual facts in support of the theory of the dependence of contagious diseases upon microscopic life had been supplied by Bassi's studies upon muscardine, but other diseases were shortly to be demonstrated to be due to similar fungi. Tulasne, De Bary, and

Kühn proved that a number of diseases of various sorts of grain, potatoes, etc., arose through the entrance and parasitic development of fungi. In man, apart from numerous discoveries of fungi which could not with certainty be demonstrated to be the cause of accompanying disease, thrush, favus, and several other skin affections were shown to be dependent upon parasitic fungi. About this time the brothers Goodsir (1842) saw in the contents of the stomach the quadratic masses to which they gave the name of sarcina ventriculi, and which Virchow believed belonged to the lower plants. Of special importance was the discovery (Pollender, 1855; Davaine, 1863) that anthrax is characterized by the presence of small, rod-shaped bodies in the blood of the animals attacked, and that these organisms could be demonstrated experimentally to be the cause of the disease.

The reappearance in Europe of cholera at the end of the fourth and the beginning of the fifth decade of this century supplied the impetus for its study by microscopic means, a study characterized by a hot-headed enthusiasm and insufficient proof, which brought little genuine support to the parasitic doctrine of disease. In the light of our present knowledge, however, it seems not at all improbable that Pouchet and Pacini had seen and described the spirillum of Asiatic cholera.

The fundamental results of the impetus given by the study of the cholera epidemic can be seen in a description of minute monads in the dejections of typhoid patients by Davaine, of several different monads in unhealthy ulcers by Wedl, of the paramecium coli in the large intestine by Malmsten, and the demonstration of numerous amœbæ in the intestinal canal of a child dead of enteritis as well as the existence of numerous cercomonades intestinales in the mucous secretions of children by Lambl, not to mention many other infusoria described as occurring in the intestinal canal by other investigators.

The intimate connection between fermentation and putrefaction and the processes concerned in contagious disease is shown in the influence which discoveries in the former fields exercised on the subsequent growth of our knowledge regarding the etiology of the latter. Before Schwann's discovery, the part played by yeast in fermentation was either entirely disregarded or was considered as only of subsidiary importance in that it behaved as a porous body, the purpose of which was to condense and transmit oxygen to other elements (sugar) in the mixture, the decomposition of which produced alcohol (Braconnot, 1831). Berzelius (1827) conceived that the action of yeast was katalyptic in nature, in that it possessed the power to set up changes in fermentable substances much in the same way as finely

divided platinum decomposes hydrogen peroxide. That fermentation was a vital process was stated by Schwann (1831) for the first time, and by him was first proved experimentally.

Experiments undertaken to prove that neither fermentation nor putrefaction sets in if all living germs have been destroyed by heat can be traced back to the earliest times. They were carried on again in this century with great vigor. F. Schulze (1836) showed that putrescible substances failed to undergo putrefaction if all contained living germs were destroyed by heat and the entrance of others was prevented by filtering the air first through sulphuric acid and then through a layer of oil which covered the heated solution. Schwann made similar observations; and Schröder and von Dusch, who confirmed them, separated mechanically the germs suspended in the air by filtration through cotton-wool. The same results, namely, the mechanical separation of suspended living particles contained in atmospheric air, was accomplished by Hoffmann, Chevreuil, and Pasteur, by causing the air to pass through glass tubes which were drawn out and bent at different angles. Solutions from which the germs had previously been eliminated by heat, when submitted to the influence of filtered air or air whose contained particles had been precipitated on the interior of glass tubes, failed entirely to undergo fermentation or decomposition. The objection that putrefaction was prevented because heating and other procedures had modified the composition of the heated substances or the air so as to render them unsuited to these changes, was met by the successful experiments of Pasteur, Rindfleisch, Lister, Meissner, Marchand, and others. They found that when various putrescible substances—grape-juice, blood, viscera—were obtained free from contaminating germs and were preserved from accidental contamination afterward, all of them remained unchanged for an indefinite period of time.

Such experiments proved beyond the possibility of doubt that fermentation and putrefaction are biological processes, depending upon the presence, multiplication, and activity of living germs, and that the exclusion or destruction of all such germs would effectually prevent the peculiar changes characteristic of these processes in substances otherwise subject to them.

The studies inaugurated by Schwann were continued and greatly extended by Pasteur, and the results of his labors may justly be considered as the foundation stones of our modern views concerning the relation of microorganisms to external nature. Cagniard de Latour and Schwann had already shown that alcoholic fermentation of solutions proceeded hand-in-hand with the development of living microorganisms, the *torula cerevisiæ* (Turpin). Pasteur proved that, just

as with alcoholic fermentation, various other natural fermentations—the lactic, butyric, and acetic—were the results of the action of certain definite microorganisms, and, moreover, that the different fermentative agents possessed not only peculiar physiological properties, but also equally definite morphological and biological characteristics.

Pasteur's studies upon fermentation were followed by similar ones dealing with putrefaction (1863). He showed that the ordinary putrefactive changes were associated with the development of "vibrios." He was therefore led to the conclusion that just as fermentation is produced by yeast, putrefaction is the result of the development of vibrios. We now know that there are many kinds of putrefaction just as there are different kinds of fermentation, and that no one organism (vibrio) is responsible for all, but that each kind of putrefaction is associated with the presence and multiplication of a particular organism or organisms.

From the study of the causes and kinds of fermentation Pasteur next turned his attention to the study of the diseases affecting wines. He was able to show that the souring and ropiness of wine were caused by particular microorganisms, the first of which developed upon the surface, forming a mycoderm, while the second appeared in the fluid as chain-like bacteria.

Next followed his famous work on pebrine, the scourge of the silkworm—an investigation which proved, in his hands, of such economic importance to France. He confirmed the observations of Cornalia, Naegeli, and Lebert, in rediscovering the oval parasitic bodies in the tissues of the developed worm, and extended their results by demonstrating the same objects both in the moth stages and in the eggs. The worms which developed from infected eggs, he found, succumbed before the spinning of the cocoons, but in the course of their existence infected the food which in turn brought about a dissemination of the disease among healthy worms. From such worms infected moths, from infected moths infected eggs, and from infected eggs congenitally diseased worms represented the cycle of events.

The great practical result of Pasteur's work on pebrine consisted in the demonstration that one could determine by the use of the microscope the uninfected eggs and separate them from the healthy ones, so that it became possible to obtain a new breed of worms free from all infection.

Notwithstanding the evidence brought to bear by Schwann, Pasteur, and others to prove the relation of microorganisms to fermentation, and the brilliant practical results of Pasteur's studies upon pebrine, which tended equally to establish the germ origin of disease,

there were not wanting scientific minds who dissented from both of these views.

One series of objections arose from the belief that fermentation and putrefaction often set in under conditions in which microorganisms were excluded. Thus, it was held that they appeared in the interiors of cadavers, in the contents of germinated eggs, in dead fœtuses of women and animals, and that in some of these instances there were found lactic, butyric, and acetic acids, just as though microorganisms had been present. Numerous attempts were made to preserve, in such a manner as to exclude germs, substances capable of undergoing putrefaction and fermentation, and yet, despite all precautions, in certain instances these changes set in (Hoppe-Seyler, Billroth, Sanderson, Paschutin, etc.). Carefully preserved urine would at times become alkaline and show putrefactive changes (Colin, Billroth, Hiller, and others); fermentation or putrefaction overtook filtered and sterile fluids (Helmholz), and fluids, heated and carbolized in order to destroy all living matter contained in them, would yet undergo these changes (Bastian, Hoppe-Seyler, et al.). Finally, Béchamp and Wigand attempted to show by numerous experiments that microorganisms are generated from dead protoplasm of higher living forms, and that in the absence of all living matter these small beings come into existence and provoke fermentative and putrefactive processes.

The germ theory of fermentation and putrefaction has not been essentially affected by these contradictory results. Increased knowledge has given us the key to many of them. The difficulty in entirely excluding germs from or destroying them in vessels and infusions explains their development in heated fluids. The differences in species, as we now know them, equally explain the varying results of their presence. Thus, for example, we are no longer surprised at the absence of perceptible changes in substances in which living microorganisms have been demonstrated; and, again, the absence of cultural tests and staining methods renders the early statements of the entire absence of germs in fermented and putrescent substances unreliable.

Of more importance was the influence of the chemical doctrine of fermentation brought forward by Liebig (1839 to 1870). He ascribed the process not to the action of living organisms, but to dissociation and recombination of the proteid molecule, due to soluble ferments contained within the yeast cell. The disintegration of the yeast cell, through which the ferment is set free, is of course a vital action, but a correlative phenomenon of its death. The difference between fermentation and putrefaction is also explicable upon this basis; in putrefaction the dissociation is brought about by the material resulting

from the decomposing albumins themselves, so that the process once begun becomes continuous even after the first impulse has ceased to operate; in fermentation, on the other hand, the sugar which is undergoing dissociation is incapable of transmitting the energy for continuous decomposition, and therefore a ferment is necessary not only for the introduction but also for the maintenance of the fermentative act.

The theory of Liebig lacked an exact experimental basis, and the one observation upon which, more than any other, it was based, namely, the self-fermentation of yeast, was shown by Naegeli to be erroneous. The last modification of the chemical theory, as stated by Liebig, looks upon the yeast cell as making and containing the ferments, and therefore holds that the production of the ferment proceeds hand-in-hand with the vital manifestations of the cell. The act of fermentation depends, however, not on the organized body—the yeast cell—but upon a ferment which this body produces, much as peptic and pancreatic cells secrete their peculiar enzymes. Were it possible to separate, in an active state, the ferment from the cell, then the latter would be no longer necessary to the process. Similar views were expressed by Traube (1858) and were elaborated later by Hoppe-Seyler. The occurrence of microorganisms in fermentative and putrefactive processes was no longer denied, but their function was regarded differently. The first changes were brought about by the ferments contained within the decomposable substances, but then, after a certain alteration had taken place, microorganisms appeared and increased. The character of the first change determined the nature of the organismal development, the germs of which, because of their wide dissemination, had gained access to the medium. The microorganisms might take part in the subsequent changes, but they were not indispensable, and, indeed, their increase did not proceed hand-in-hand with the process of decomposition.

Although the rôle of the chemical ferments was never for a moment questioned by the advocates of the germ theory, on closer examination it is found that the products of decomposition, caused on the one hand by them *per se* and on the other hand through the agency of living organisms, are quite different. The chemical ferments bring about hydrolytic dissociation; their place can often be taken by certain chemicals, acids, and alkalies; the quantity of the ferment either remains the same or it diminishes as the process goes on; the optimum temperature for their operation is around 60° C., and the exquisite physiological poisons fail to affect them injuriously. In fermentation and putrefaction, on the other hand, the products of decomposition and putrefaction are complex. Carbon dioxide and

perhaps other atomic groupings are split off, the quantities of the active organisms increase proportionally to the intensity of the fermentation; their activity is greatest from 25–40° C., and under the influence of physiological poisons ceases altogether (Gotschlich, in Flügge's "Microorganismen," 1896).

If in the last instance it should be admitted that Liebig is right in attributing the atomic decomposition and rearrangement to ferments produced by living microorganisms—which in view of Edward Buchner's results in obtaining from yeast cells through the use of great pressure a ferment capable of transforming sugar into alcohol and carbon dioxide seems not improbable—this fact cannot be regarded as militating against the germ theory of fermentation and putrefaction. Indeed such a view if true would form an additional support for the germ theory, inasmuch as the production of the ferment depends upon the vital activities of cells; and proof of such a mode of action would merely extend and deepen our knowledge of the manner in which the organisms bring about the complex changes associated with their growth and multiplication.

The experiments of Lemaire are important as indicating how by an entirely new line of work conclusions similar to those of Pasteur were arrived at. He found that fermentations of the ordinary character were rendered impossible if a certain proportion of carbolic acid were added to the infusion; whereas the changes set up by the unorganized ferments—diastase, myrosin—were in no way influenced by this agent. From these observations he drew the conclusion that there were two kinds at least of fermentation; one the result of the action of living organisms, the other the result of the action of non-organized ferments. His further studies led him to similar conclusions concerning the causes of miasmatic disease. He found that fluids capable of producing upon inoculation pustule formation and suppuration were likewise deprived of this power by the addition of carbolic acid. He therefore concluded that both fermentation and the contagious diseases resulted from the action of living organisms.

In the course of its development the doctrine of the parasitic nature of contagious disease struggled at all times against detractors, just as the similar doctrine of the germ or vital origin of the processes of fermentation and putrefaction had been compelled to do. It has also happened that some of the supporters of the doctrine have by their misdirected enthusiasm exercised the most hurtful influences. Thus Hallier (1866–68) asserted that the various microorganisms were only special forms of moulds which, having arisen through peculiar external conditions of life, gave rise to all kinds of disease; but that under suitable conditions one could always cultivate from

any particular disease a corresponding mould and in this way demonstrate its true etiological factor. Through the study of diseased organs and excreta Hallier obtained a fungus appearing under a variety of different forms which he proclaimed to be the causative agents, and in a short time scarlet fever, measles, as well as cholera, typhoid fever, and still other diseases were referred to this supposed origin.

Criticism of this phantastic idea was of course inevitable. Authorities on fungi such as De Bary showed Hallier's investigations to be worthless, because they had not been conducted with care sufficient to exclude the entrance of extraneous organisms. De Bary's views were confirmed and the structure of Hallier's teaching on parasitic disease fell, and with it a serious blow for a time was given to the whole parasitic theory.

Further undoubted discoveries of parasites made in the next few years tended to restore this lost confidence. This was especially the case with the wound infections. Rindfleisch, Waldeyer, and von Recklinghausen were the first to direct attention to the regular occurrence of minute organisms in pyæmic processes. Other observations of a similar nature were made by Hüter, Orth, Oertel, and others in erysipelas, phlegmon, and puerperal fever. Finally, the pathogenic nature of the organisms described was shown by experiments on animals (Flügge).

The most important microorganism to which Hallier drew attention was the *micrococcus*, which on account of its small size he believed to be especially adapted for entering the capillary vessels. Following his observations and the study of the nature of vaccine by Chauveau, a whole host of publications appeared concerning the relation of cocci and vibrios to a great variety of pathological processes in man and animals. Among others Buhl, Oertel, and Nassiloff were able to show that in diphtheria such cocci were present not only in the false membrane but also in the lymph vessels of the submucosa, the lymphatic glands, and the internal organs. Mayrhoffer described vibrios in the secretions from the uterus in puerperal fever; Pouchet a variety of bacteria in bronchitis; while Leyden and Jaffé proved that in putrid bronchitis and gangrene of the lung the sputa obtained from the diseased organs contained large numbers of bacteria and spirilla. Traube pointed out that the severe inflammations of the bladder following catheterization depended upon the introduction of microbes into the bladder upon the catheter, and Klebs referred pyelonephritis to the emigration of similar germs from the bladder into the ureters and the uriniferous tubules.

About this time (1867–69) Rindfleisch showed that pyæmic ab-

scesses in the heart wall were filled with vibrios rather than with pus corpuscles. A more accurate contribution was made by von Recklinghausen (1871), who proved that in a whole series of infectious diseases, but especially in pyæmia and puerperal fever, there occurred aggregations of micrococci in the primarily diseased areas, and that these micrococci existed also in the metastatic foci. He pointed out that these bodies possessed great resistance to chemical reagents, and that they were so uniformly granular that it was impossible for them to be confounded with ordinary tissue detritus. He regarded the bodies as probably identical with those described by Buhl, Oertel, and others in diphtheria, and by Klebs in cystitis and pyelonephritis.

In the same year Waldeyer confirmed these observations, having himself examined pyæmic areas in the muscle of the heart and suppurative processes in the kidney. Shortly after came the observations of Weigert upon the pustules in smallpox, in which he demonstrated micrococci similar to those described by von Recklinghausen, and also showed that the lymphatics of the skin were filled with these bodies. Klebs studied a large number of infected wounds in soldiers engaged in the Franco-Prussian war of 1870–71, and found constantly in the secretions from them, as well as in the organs of those who had succumbed to septic and pyæmic processes, certain bacteria, some appearing as rods and others as cocci. Klebs confounded these several organisms, believing, in keeping with Hallier's scheme, that he was dealing with a single pleomorphic form to which he applied the name microsporon septicum.

The discoveries of Lister in the surgical treatment of wounds exerted a markedly beneficial influence in securing for the parasitic theory a wider acceptance. Although the opponents of the doctrine had gradually diminished in number and scepticism in its validity was certainly disappearing, yet the methods which had thus far been employed were not such as to place the theory upon an impregnable foundation. There still was great need for a more complete and accurate study of the microparasites of disease. The researches of Pasteur, Cohn, Koch, Brefeld, Naegeli, Weigert, Ehrlich, Kitasato, Behring, Roux, and many others have supplied the desired support.

These later studies have given us a knowledge of the life history of the lowly microorganisms; they have established the fixity of species; and by providing ready and accurate methods of isolation, cultivation, and demonstration of microorganisms, have taught us how to distinguish the injurious pathogenic from the innocuous saprophytic forms, to obtain in solution the active products of their growth

and to discriminate the part played by these substances from the mere mechanical effects of the organisms themselves.

Thanks to the brilliant work of Koch, the isolation of organisms by the use of solid media and of pathogenic species by the use of the animal body opened up a ready method of separating mixtures of species and of obtaining pure cultures. The successive cultivation outside the animal body of pathogenic microorganisms to scores or hundreds of subcultivations and the reproduction of disease by the inoculation of infinitesimal amounts of such cultures successfully disposed of the objection that the pathological condition may have been caused by some other body mechanically admixed with the organisms. This principle, first introduced by Pasteur and Klebs and so greatly extended by Koch, brought the final proof of the relation of definite and specific organisms to infectious and contagious disease. It was found that the inoculation of the smallest quantities of subcultures removed a hundred times or more from the original sources of anthrax, the septicæmias, tuberculosis, etc., sufficed to reproduce the typical diseases with their classical symptoms and pathological alterations, while the organism introduced was capable of recultivation from the pathological condition thus set up.

A still further advance in our knowledge of bacteria resulted from the introduction by Weigert of the aniline colors as staining agents. In 1875 this investigator first applied methyl violet to the study of tissues containing bacteria, and in 1877 he was able to show preparations from many organs, stained in different aniline dyes, in which the contained microorganisms were rendered distinctly visible. Koch modified and extended the use of staining agents by applying them to thin films of bacteria dried upon cover-slips which afforded the sharpest pictures and greatly aided the study of morphology.

Through these and other studies, the results of which are given in the special chapters, the doctrine that minute organisms act as parasitic exciting agents of disease has been placed as much beyond question as is the function of similar minute beings in exciting fermentation and putrefaction. A somewhat new direction has been given to the study of the relation of microorganisms to disease through the production of artificial vaccines and the increasing importance attaching to the action of their metabolic processes. The influence which the study of the living disease germs themselves, and later of the poisonous products of their growth, has had upon the progress of medical science and especially upon therapeutics, is shown by the application of the principle of antitoxic treatment of morbid processes to several typical infective diseases, among which may be mentioned diphtheria, tetanus, streptococcus infection, and that allied condition,

snake-poisoning, as well as the employment of artificial vaccines for the protection of domestic animals and man from such fatal scourges as anthrax, chicken cholera, Asiatic cholera, and bubonic plague.

The demonstration of the causal relation of microorganisms to disease, now completed, has been succeeded by an era in which the chief attention is being directed to the elucidation of the manner of action of the organisms, of the processes upon which their peculiar effects depend, and of the methods by which nature combats the invasion and ultimately expels the enemy. This quest has resulted in the discovery by Roux and Yersin, Brieger, Fraenkel, Ehrlich, Kitasato, Behring, and others, of a large and important group of poisons —ptomains and toxins—upon which, in large part, the activities of the parasites depend. But, furthermore, it has been discovered that toxins undergo in the animal body transformations, perhaps indirectly through changes brought about in the somatic cells, by which are produced antidotes—antitoxins—to the original toxic substances generated by the germ.

Though not a little has already been accomplished, much still remains to be done. The etiology of the most exquisite of the contagious diseases—the exanthemata—still remains unsolved. With our present knowledge we can say that the discovery of parasites in morbid states can be regarded as bearing on the question of the causation of diseases only if certain fundamental conditions have been fulfilled. The wide dissemination of microorganisms, the rigid precautions required to prevent effectually their accidental presence, and the accurate and delicate means at our command for demonstrating their occurrence, have necessitated the employment of a rigid and inflexible scrutiny before accepting as the cause of disease any given microorganisms and, further, have justified the postulates of Koch which provide:

1. That any given microorganism shall be found constantly in the diseased individual and in such relation with the pathological process as to suggest a causal connection with that process.

2. That the microorganism shall not be present under normal conditions and in the normal state.

3. That the microorganism shall be obtained in culture uncombined with other organisms and shall be grown in subcultivations so as to remove any extraneous substance which may have been present in the material from which the original culture was obtained.

4. That upon the inoculation of such subcultures into susceptible animals the same pathological condition shall be set up, and that from the lesion it shall be possible to recover the organism introduced.

To these postulates may be added still another based upon the

observations of Pfeiffer, Widal, Durham, Gruber, and others, that when a bacterium has caused a pathological state in man or animal, the blood serum and some other body fluids will cause cultures of the organism to undergo agglutinations, while it is without effect on other even closely related forms. Therefore,

5. Cultures from the microorganism in question should, when tested with the blood serum of the diseased individual, in a proper state of dilution, undergo those changes in arrangement to which term *agglutination* is applied.

Classification of Microorganisms.

By microorganisms are meant minute living beings which belong partly to the animal and partly to the vegetable kingdom. Owing to the difficulty in placing accurately the limits of these two great classes, some biologists (Haeckel and others) have suggested the existence of a third kingdom of living things which it is proposed to designate protista, in which the differentiation into animal on the one hand and vegetable on the other has not been completed. The protista would embrace the protozoa from among the microorganisms now regarded as of animal nature, together with the cyanophycaceæ (blue-green algæ), and some of the simple green algæ and fungi from among those now considered as vegetable. But even accepting this innovation, the line of demarcation between protista and animals or vegetables would still be arbitrary, so that any advantage to be derived from the introduction of the new class is theoretical only and would not amount to much more than an admission of our imperfect knowledge of the ultimate nature of these minute beings.* Microorganisms, a term which is generally taken to include the lower fungi (moulds or hyphomycetes, yeasts or blastomycetes, bacteria or schizomycetes, streptothrices) and the protozoa, may, it is true, in part be grouped with the protista; but this would be of doubtful advantage in our present state of knowledge, and the commonly accepted division into animal and vegetable organisms, in which the protozoa are considered as of animal, the others as of vegetable nature, can properly be employed at this time. In this article only those microorganisms (microbes) which are of medical interest and related to pathological conditions in man and the higher animals will be considered, and it may be said at the outset that while certain representatives of the several groups mentioned are associated with pathological states, the chief place in this connection must be assigned to the bacteria. The protozoa will be treated of in a separate article.

Bacteria.

Bacteria were considered by Ehrenberg as very complex infusoria; later students, on the other hand, regarded them as simple plants consisting merely of protoplasm and cell membrane. In point of fact, as we now know, only the simplest bacteria are composed of homogeneous protoplasm. A. Fischer[3] has shown that bacterial protoplasm reacts like vegetable protoplasm and that it usually contains paraplastic granules resembling fat, starch, or sulphur. Vacuoles are sometimes present in the protoplasm. The cell membrane is usually of a nitrogenous nature and consists of physiologically altered protoplasm; some forms, however, possess cellulose-like membranes and approach, therefore, more nearly the higher plants.

Hueppe[4] has drawn attention to the alterations which take place in the bacterial cell during division. The chromatic granules undergo a rearrangement of a definite nature, which he has compared with the appearances seen in the chromosomes in the dividing higher cell. Bacteria in general stain with nuclear dyes. Bütschli believes that the entire central part of the cell corresponds to the nucleus of higher cells. According to this idea the smaller bacteria consist almost entirely of nuclear substance. Other structures, presenting the staining properties of nuclei, although differing from them markedly in morphology, have been described by Ernst, Babes, Metchnikoff (metachromatic bodies), and others. These structures which may appear with great regularity, often at the poles or in the centre of the cell, are believed by A. Fischer not to be preformed organs but to result from plasmolysis due to the action of reagents. According to this view the nuclei of Bütschli are merely contracted or plasmolyzed protoplasm.

Bacteria have been classed with the fungi. In their mode of life fungi and bacteria are in agreement. Excluding the nitrifying bacteria and a few others, neither are capable of deriving subsistence from inorganic bodies. They are therefore metatrophic (Fischer), that is, they are dependent for their nutriment upon those organic compounds elaborated by the more highly organized animals and plants. A special group of bacteria is capable of growing and multiplying only within another organism (parasitism); they are paratrophic. Yet, notwithstanding this agreement, there are also points of difference. The distinction in the fungi of vegetative and reproductive organs is wanting in the bacteria whose vegetative elements consist of single cells or groups of cells, while no special reproductive organs exist in them. In the production of spores in the bac-

teria either the whole vegetative cell becomes converted into conidia as in cladothrix, or during the formation and development of the spore the vegetative cell as such ceases to exist.

The bacteria may be divided into groups according to the several forms under which they tend to appear in nature or as a result of artificial cultivation. These characteristics do not necessarily indicate relationship, but as they are, under given conditions, constant, they facilitate the rapid distinction of form. Again, as certain forms vary in morphology within limits which are known or can quite readily be discovered, these in turn are found to afford certain criteria for the distinction of genera and species. The general groups based upon morphology are:

1. *Cocci.*—These consist of spherical or ellipsoidal cells, which appear singly, united into chains, arranged in definitely numerical groups, or conglomerated into more or less irregular masses. The terms diplococci (pairs), streptococci (chains), tetrads or meristaforms (packets of four), sarcinæ (packets of sixteen or more), and staphylococci (grape-like clusters) are employed to designate the different natural groupings.

2. *Bacilli.*—Elongated cells whose length definitely exceeds their breath. Distinctions into long and short rods are made. Most of the rods have a uniform diameter, while some few show irregular swellings or thickenings. Descriptive names are applied to these irregular forms, such as hetatone, spindle-shaped, club-shaped, etc. The ends are sharply contoured and either square or round; more rarely they are pointed. The rods may be rigid or motile, in the latter case appearing at times more or less bent. In the course of division and growth the rods may separate, remain united in pairs (diplobacilli), or form a more elongated chain (streptobacilli); or the lines of separation may temporarily be lost, when pseudo-threads arise.

3. *Vibriones.*—This group includes all spirally twisted bacteria. The individuals may appear as complete spirals or in the smaller forms as slightly curved or comma-like bodies. They may be rigid or undulating and of uniform or irregular diameter.

The variations met with within these great groups are not greater than those found in other organisms. According to the condition of growth—temperature, nutriment, etc.—alterations in size are met with, but the form-types nevertheless remain constant. Cocci cultivated indefinitely appear as spherical or ellipsoidal bodies, and bacilli and vibriones are equally constant. All bacteria tend to undergo involution when they are compelled to remain for a long time under unfavorable conditions of growth. Evidences of involu-

tion are seen constantly in old artificial cultivations, and in those conducted at unfavorable temperatures or in unfavorable media. These evidences consist in the appearance of irregular, badly-staining, and misshapen individual organisms, which often do not present the remotest resemblance to the normal forms obtained under more favorable conditions of growth. When involution has reached its height, the bacteria are no longer capable of multiplication if they are again brought under the conditions suitable for the species in the normal state. They have succumbed and have lost their ordinary vitality forever.

The bacteria are separable into natural genera and species. The erroneous views of Billroth, according to which all bacteria occurring in infected wounds represent only stages in development of one natural species—the *coccobacteria septica*—and the curious speculations of Zopf, who imagined that a given bacterial species in the several stages of development can pass through a variety of forms, hitherto regarded as types of different genera, have now merely an historical interest. Yet, notwithstanding the belief that the bacteria form genera and species, the morphological criteria at our disposal for these distinctions are so scanty that we are forced to call to our aid the help of certain physiological features. These characteristics in part are (1) The manner of growth upon various culture media; (2) the formation of specific products, such as pigment granules, iron, sulphur, light; (3) the capacity to set up certain biological processes, such as fermentation, putrefaction, disease; and (4) the relation of the organisms to oxygen. It is self-evident that a classification based upon physiological phenomena is more or less arbitrary and fails to take into account natural affinities. For medical purposes, however, such a classification has fewer objections than from the standpoint of the botanist. Indeed, for the present, a classification by which pathogenic bacteria are grouped according to (1) morphology, (2) culture affinities, and (3) pathological effects, though artificial, serves the most useful if not the most rigidly scientific purpose.

Beginning with Ehrenberg's publication, various attempts have been made to construct a system of classification for microorganisms. Ehrenberg (1838) placed the bacteria with the infusoria under the special designation of vibrionia which he subdivided into five genera:

1. Bacterium: Straight and rigid threads (three varieties.)
2. Vibrio: Straight threads; serpentine motility (six varieties).
3. Spirochæte: Bendable spiral threads (one variety, plicatis).
4. Spirillum: Rigid spiral threads (three varieties).
5. Spirodiscus: Rigid and screw-like. Seen but once.

Davaine (1868 and 1869) suggested the accepted name of bacteria

for the class and designated the non-motile forms as bacteridia. Hoffmann (1869) brought the spherical elements, which before had been classed with the monads, into relationship with the bacteria under the name, first employed by Hallier, of micrococci. Billroth regarded these several forms as representing only the different stages of development of a single species, the coccobacteria septica. According to the mode of grouping Billroth spoke of mono-, diplo-, glia-, petalo-, and asco-cocci and bacteria. Some of these terms are still in common use.

The first classification which may be regarded as an attempt to found a natural system is that of F. Cohn (1872). The chief criteria employed in his scheme were the presence or absence of zooglœa and the production of threads. Cohn distinguishes four great families of bacteria and a variable number of genera in each family.

Family I. Sphærobacteria: spherical or oval; non-motile; tend to grow into zooglœa.

Genus 1. Micrococcus { Chromogenic varieties. / Zymogenic " / Pathogenic "

Family II. Microbacteria: short rods; motile; tend to produce zooglœa.

Genus 1. Bacterium: { B. termo. / B. lineola.

Family III. Desmobacteria: Thread-like forms; no zooglœa.

Genus 1. Bacillus: { B. subtilis / B. ulna / B. anthracis } Straight threads.

Genus 2. Vibrio: { V. rugula / V. serpens } Threads bent.

Family IV. Spirobacteria: Motile spirals; no zooglœa.

Genus 1. Spirochæte (S. plicatilis). Flexible, long, convoluted spirals.

Genus 2. Spirillum { S. tenue / S. undula / S volutans } Rigid, short, and wider spirals.

In 1875 Cohn brought the ordinary bacteria into relationship with the phycochromaceæ (chlorophyll-containing algæ) and the sulphur and purpuric bacteria. In this later scheme we find the terms leptothrix, crenothrix, cladothrix, and streptothrix. Zopf (1883–85) again separated the bacteria from the algæ (schizophytes), where they

had been placed by Cohn, and classed them with the fission fungi (schizomycetes). He recognized four families:

Family.	*Genus.*
I. Coccaceæ. Consist of spherical cells.	1. Streptococcus. 2. Merismopedia. 3. Sarcina. 4. Micrococcus. 5. Ascococcus.
II. Bacteriaceæ. Consist of cocci, straight or bent rods and straight or spiral threads.	1. Bacterium (cocci and rods; no spores). 2. Spirillum (spirals without spores). 3. Vibrio (spirals with spores). 4. Leuconostoc (cocci and rods in chains; spores; zoogloœa). 5. Bacillus (cocci and rods; spores). 6. Clostridium (like the bacilli but with spores in spindle-shaped forms).
III. Leptothricees. Appear as cocci, rods, and threads (straight or spiral) with distinction of base and apex. No spores.	1. Crenothrix (with membrane; no sulphur). 2. Beggiatoa (without membrane; contains sulphur). 3. Phragmidiothrix (without membrane or sulphur; very extensive division). 4. Leptothrix (no sulphur; membrane variable; slighter division).
IV. Cladothricees. Appear as cocci, rods, threads, and spirals. Threads show pseudo-branching. No spores.	1. Cladothrix (includes streptothrix).

The system of Zopf shows clearly his belief in the common pleomorphism of bacteria. Those forms which showed the greatest variation are leptothrix, beggiatoa, crenothrix, phragmidiothrix, cladothrix, and streptothrix. The studies of Winogradsky have shown a pleomorphism far less extensive than Zopf and his immediate followers had assumed. Beggiatoa and cladothrix have been proven by Winogradsky to possess relatively narrow limits of variation. The simultaneous occurrence of several morphological forms of bacteria is no proof of pleomorphism; in most instances it is an indication of mixture of species and impurity of culture. "Leptothrix buccalis" is certainly an example of such a mixture; and streptothrix has been shown to be a widely disseminated and well characterized group, which, while resembling the bacteria in form and size, exhibits branchings similar to the hyphomycetes.

The production of spores, in opposition to the utilization of

purely morphological characteristics, has been used as the basis of classification, first by Van Tieghem (1883), then by De Bary (1884), and more particularly by Hueppe.[4] In its most fully developed form this system, as given by Hueppe, presents:

Family I. Coccaceæ; in vegetative stages produce coccus forms.

Genus 1. Micrococcus; characterized by irregular arrangement of cells and cell groups; endospores at present unknown.

Genus 2. Sarcina; appears as threads and packets of cells; endospores certainly known.

Genus 3. Streptococcus; produces chains; arthrospores definitely known; endospores doubtful.

Family II. Bacteriaceæ; in vegetative stages form rods which may become twisted into chains or pseudo-threads.

Genus 1. Arthrobacterium s. Bacterium; forms arthrospores.

Genus 2. Bacillus; forms endospores.

Subgenera: (*a*) Bacillus; has straight rods.
(*b*) Clostridium; has spindle-shaped rods.
(*c*) Plektridium; has hammer-like rods.

Family III. Spirobacteriaceæ; form in vegetative stages short spirals (comma and *S* forms) which may grow into spiral pseudo-threads.

Genus 1. Spirochæta; no endospores, but with arthrospores.

Genus 2. Vibrio; with endospores; the spiral alters its form in process of sporulation.

Genus 3. Spirillum; with endospores; the spiral does not alter its form in sporulating.

Family IV. Leptothriceæ; in the vegetative stages form rods which unite into threads.

Genus 1. Leptothrix; distinguished from the pseudo-threads of the arthrosporic bacteria in that the threads show a definite apex and base.

Genus 2. Beggiatoa; the threads have no membrane and the cells contain sulphur granules.

Genus 3. Phragmidiothrix; the threads are united into short, cylindrical elements, which may break up into semicylindrical and cylindrical bodies and ultimately into spheres.

Genus 4. Crenothrix; the threads possess a membrane and usually with a deposit of iron granules.

Family V. Cladothriceæ; the vegetative cells belong to the bacilli, but the rods give rise to branches and are enclosed in a membrane.

Genus 1. Cladothrix.

The great uncertainty which surrounds the so-called arthrospores, the nature of which is claimed by many bacteriologists to be un-

known, makes any system based upon these structures of very doubtful value.

The rapid progress of bacteriological discovery, following the brilliant researches of Pasteur and Koch, and the introduction by the latter of ready means of isolating, in pure forms, numerous bacteria, soon made evident the insufficiency of the system constructed by Cohn, as well as of those modelled upon it. The important relation of bacteriology in its later developments to medical science led Flügge in his celebrated work[2] to adapt the system of Cohn to the uses of medical bacteriology. But while adhering in the main to this classification, Flügge has not hesitated to introduce large numbers of new species. He believed in the persistence and independence of the three great form groups as represented in the cocci, bacilli, and vibriones; while in respect to the filamentous bacteria he agreed with Zopf in considering them pleomorphic. Within individual genera Flügge accepted physiological differences as sufficient to distinguish species, without, however, committing himself to the belief that such distinctions need necessarily imply natural differences.

In the efforts made to bring a modicum of order into the rapidly developing chaos of bacterial species, physiological criteria came to assume a very undeserved importance. This is shown in the work of Eisenberg,[5] in which classification upon this basis alone has been attempted. Eisenberg separated all known bacteria into (1) non-pathogenic and (2) pathogenic forms. These he subdivided as follows:

I. Non-pathogenic Bacteria.
  (1) Micrococci.
    A. Liquefy gelatin.
      (*a*) Produce pigment.
      (*b*) Do not produce pigment.
    B. Do not liquefy gelatin.
      (*a*) Produce pigment.
      (*b*) Do not produce pigment.
  (2) Bacilli.
    A. Liquefy gelatin.
      (*a*) Produce pigment.
      (*b*) Do not produce pigment.
    B. Do not liquefy gelatin.
      (*a*) Produce pigment.
      (*b*) Do not produce pigment.
  (3) Spirilla.
    A. Liquefy gelatin.

(*a*) Produce pigment.
(*b*) Do not produce pigment.
B. Do not liquefy gelatin.
(*a*) Produce pigment.
(*b*) Do not produce pigment.
II. Pathogenic Bacteria.
(1) Specifically pathogenic for man.
(2) Specifically pathogenic for animals.
(3) Pathogenic for animals, but found in man.
(4) Pathogenic for animals, but of various origins.

Such a system must of course be entirely artificial and arbitrary. It has, however, served a useful purpose especially in respect to the pathogenic forms, while it has at the same time emphasized certain physiological characteristics possessed by bacteria.

Other, larger and more complex systems are those of De Toni and Trevisan based upon morphological, and that of Miguel upon physiological criteria. They do not represent advances in our knowledge.

More recently (1894) Migula[6] has formulated a system of which the following is the scheme:

Bacteria.

Phycochromeless fission plants which divide in one, two, or three dimensions of space. Many species possess endospores; when motility is present it is brought about through the agency of flagella, or somewhat rarely by means of an undulating membrane (transition to the phycochromaceæ).

Family I. Coccaceæ.

Cells which in the free state are completely spherical; division in one, two, or three dimensions of space in that each sphere divides into halves, quarters, or eighths, each of which grows into a perfect sphere. Endospores rare.

Genus 1. Streptococcus. Cells which divide in one direction in space, whereby, the cells remaining united, bead-like chains come to be produced. Organs of locomotion not present.

Genus 2. Micrococcus. Cells divide in two directions, in which case, the cells remaining united, merismopedia-like clusters are formed. No organs of locomotion.

Genus 3. Sarcina. Cells which divide in three dimensions. The cells remaining united, bale-like packets result. No organs of locomotion.

Genus 4. Planococcus. Cells divide in two dimensions as in micrococcus, but they possess flagella.

Genus 5. Planosarcina. Cells which divide in three dimensions, but possess flagellated organs of locomotion.

Family II. Bacteriaceæ.

Long or short cylindrical cells which appear as straight, never as spiral, rods; division in one dimension of space after growth in length.

Genus 1. Bacterium. Cells without organs of locomotion; endosporic.

Genus 2. Bacillus. Cells surrounded by flagella; often possess endospores.

Genus 3. Pseudomonas. Cells with polar flagella. Endospores rare.

Family III. Spirillaceæ.

Cells which are spirally twisted or which represent sequents of such spirals. Division, after growth in length, in one dimension of space.

Genus 1. Spirosoma. Cells rigid and without locomotive organs.

Genus 2. Microspira. Rigid cells with one, more rarely with two or three polar, wavy flagella.

Genus 3. Spirillum. Rigid cells with polar brushes consisting of from five to twenty, mostly hemispherical or very flat undulating flagella.

Genus 4. Spirochæte. Serpentine cells. No locomotive organs, perhaps possess an undulating membrane.

Family IV. Chlamydobacteriaceæ.

Forms of very various stages of development and characterized by the presence of a firm capsule or membrane which surrounds the cell and any branches which may be present.

Genus 1. Streptothrix. Simple, non-branching threads. Division in one dimension in space. Multiplication by means of non-motile conidia.

Genus 2. Cladothrix. Cells exhibiting pseudo-branching threads. Divide in one direction in space. Vegetative increase through separation of complete branches. Multiplication by means of polar flagellated swarming spores.

Genus 3. Crenothrix. Cells united into non-branching threads. At first division in one dimension, later in three dimensions in space. Products of division become rounded and act as germinal cells.

Genus 4. Phragmidiothrix. Cells which in the beginning appear as unbranched threads; divide in three directions in space and thus give rise to a cell strand. Later separate cells may grow through the fine, closely applied membrane and thus give rise to branching.

Genus 5. Thiothrix. Unbranched, membranous, non-motile

threads; division in one dimension in space. Cells contain sulphur granules.

Addition. Family V. Beggiatoaceæ.

Membranous cells united as threads. Division in one direction in space. Motility by means of undulating membrane as in oscillaria.

Genus 1. Beggiatoa. Cells contain sulphur granules.

This system is based upon (1) the mode of increase and reproduction; (2) the means of locomotion and the character of the locomotive organs. It is of course questionable whether such morphological criteria as possession, number, and arrangement of flagella are sufficiently definite to permit of their use in discriminating genera. Our present knowledge is insufficient to answer this question in the affirmative. A great drawback to the system of Migula consists in the employment of terms of established meaning, while giving them a new signification, as for example, bacterium, spirillum, streptothrix, etc.

Messea has proposed the division of the bacteria into two great groups upon the basis of non-flagellation or flagellation:

I. Gymnobacteria (possessing no flagella).

II. Trichobacteria.

The trichobacteria are subdivided according to the number and arrangement of their flagella into:

1. Monotricha (one polar flagellum).
2. Lophotricha (bundle of cilia at one pole).
3. Amphitricha (a flagellum at each pole).
4. Peritricha (flagella surrounding the bacteria).

The insufficiency, if not the artificiality, of this classification needs no comment.

A. Fischer, in attempting to establish a natural system of classification, has made use of the several distinctive morphological characters possessed by the bacteria. He would insist first upon a distinction between the filamentous bacteria and all others which consist of a single vegetative cell. The thread-like (filamentous) bacteria are distinguished as *trichobacteria* and all other forms as *haplobacteria*. According to Fischer, the use of the organs of motility as a morphological differential characteristic is fully justified, and he further believes that the constancy and uniformity of this feature has been underestimated. A second fundamental and uniformly characteristic feature he finds in the manner of sporulation. Thus, the anthrax bacillus during sporulation does not lose its cylindrical form, while the tetanus bacillus assumes a hammer form (plectron), and the butyric-acid bacillus becomes constantly spindle-shaped. As regards those bacteria in which spores are thus far unknown, the rule of arranging the well-known forms in definite genera and placing the others

provisionally in these groups, is followed. The names of the genera are derived from the form of the sporulating rod and the mode of flagellation, the first supplying the prefix, the latter the suffix. Thus monotricha is represented by *inium*, lopotricha by *illum*, and peritricha by *idium*. Rod becomes *baktron*, spindle *closter*, and hammer *plectron*. The cocci are distinguished according to their mode of division. In the *homococcaceæ* the successive divisions are sharp and definite; in the *allococcaceæ* no such regularity exists.

Order 1. Haplobacteriaceæ. Vegetative bodies, single, spherical, cylindrical, or spiral. Occur singly, joined into chains, or in other vegetative forms.

Family I. Coccaceæ. Spherical bacteria. Vegetative forms, spherical.

Subfamily 1. Allococcaceæ. Variable mode of division; no sharply differentiated vegetative forms. Occur in short chains, grape-like clusters, pairs or singly.

Genus: Micrococcus Cohn. Non-motile.

To this genus belong the larger numbers of spherical bacteria, including staphylococcus and gonococcus.

Genus: Planococcus Migula. Motile.

Subfamily 2. Homococcaceæ. Definite mode of division for each kind.

Genus: Sarcina Goodsir. Division in three dimensions in space with the production of packet-like vegetative forms. Non-motile.

Genus Planosarcina Migula. Same as foregoing; motile; monotrichous.

Genus: Pediococcus Lindner. Division in a crucial direction and in two dimensions in space. Cells united in four parts or packets. To this genus belong the micrococcus tetragenus, the sulphur bacterium, thiopedia, and others. Perhaps also certain forms grouped as ordinary micrococci.

Genus: Streptococcus Billroth. Division parallel and always in the same direction; grows in chains. To this genus belong the streptococcus of medical literature and leuconostoc.

Family II. Bacillaceæ; rod-shaped bacteria. Vegetative forms, cylindrical, ellipsoidal, oval, and straight. The short and almost spherical forms differentiated with difficulty from cocci; division always at right angles to the long axis.

Subfamily 1. Bacillaceæ. Sporulating rods unalterably cylindrical.

Genus: Bacillus Cohn. Non-motile.

To this genus belong B. anthracis, B. tuberculosis, B. diphtheriæ, and many others.

Genus: Bactrinium A. Fischer. Motile; monotrichous, with one polar flagellum.

In this genus are for the present to be reckoned all monotrichous rod-like bacteria whose spores are unknown; for instance, B. pyocyaneus.

Genus: Bactrillum A. Fischer. Possesses lophotrichous flagella. For the present many forms without spores, as B. cyanogenus of blue milk, are here assigned.

Genus: Bactridium A. Fischer. Motile; peritrichous; for the present includes asporogenous forms also; among the sporulating bacilli, B. subtilis and B. megatherium; among the asporogenous, B. proteus, typhi, coli, etc.

Subfamily 2. Clostridieæ. Sporogenous rods spindle-shaped.

Genus: Clostridium Prozmowski. Motile, peritrichous. To this genus belong several butyric-acid bacteria.

Subfamily 3. Plectridieæ. Spore-forming rods, hammer-like.

Genus: Plectridium A. Fischer. Motile, peritrichous. To this genus belong several butyric-acid bacilli, some methane bacteria and B. tetani.

Family III. Spirillaceæ. Spiral bacteria. Vegetative forms cylindrical, but spirally bent; division at right angles to long axis.

Genus: Vibrio Müller-Löffler. Slightly comma-like; motile; monotrichous: V. choleræ asiaticæ and numerous forms from fresh and salt water.

Genus: Spirillum Ehrenberg. More spirally bent in wider curves; motile; lophotrichous. S. undula, rubrum, etc.

Genus: Spirochæte Ehrenberg. Very short but with numerous curves; flagella unknown. Cell wall probably flexible. Spirochæte Obermeieri.

Order 2. Trichobacteriaceæ. Vegetative form is an unbranched or branched filament, whose members separate as swarming cells (Gonidia).

Family I. Trichobacteriaceæ. Filamentous bacteria.

(*a*) Filaments non-motile; rigid; enclosed in a membrane.

Genus: Crenothrix Cohn. Threads unbranched; no sulphur.

Genus: Thiothrix Winogradsky. Same as foregoing, but contains sulphur.

Genus: Cladothrix Cohn (incl. of sphærotilus); filamenta unbranched; pseudo-dichotomous.

(*b*) Pendulous thread, with slow, crawling motion; no membrane.

Genus: Beggiatoa Trevisan. Contains sulphur. To set apart a special genus for the streptothrices is regarded by Fischer as an unjustifiable assumption. Indeed, he believes that streptothrices are

only special forms of hyphomycetes which in our artificial cultures grow as sterile mycelia, or give rise to chains of segments which are located upon the mycelia and correspond to the conidia of the true fungi.

The foregoing systems of classification, while attempting, with greater or less success, to bring into relation natural affinities, must of necessity be merely provisional. The imperfect manner in which many of the special characteristics (flagellation, sporulation) have been worked out is sufficient evidence for this. It is to be regretted, also, that there is not more agreement as to the significance of terms —*e.g.*, streptothrix and cladothrix—which, in the different systems, may be applied to diametrically different forms. For the purposes of the bacteriologist as opposed to the botanist the systems described leave much to be desired; and in the present state of our knowledge it seems more important to take account of physiological rather than of mere morphological characteristics, and, as far as possible, to balance the two sets of phenomena one against the other, using both to form groups where the morphology and life history and physiological properties seem to indicate affinity. The system employed by Kruse (in Flügge's "Die Microorganismen," Auflage II.) takes account of (*a*) morphology; (*b*) mode of division and growth; (*c*) pathogenicity; (*d*) metabolism, pigment production, peptonizing power, storing up of sulphur and iron granules, etc.; (*e*) aerobiasis and anaerobiasis; (*f*) sporulation; (*g*) behavior with Gram's stain. This classification distinguishes:

I. Coccaceæ: Cocci.

A. *Streptococcus:* Growth in one dimension of space.

1. Saprophytic streptococci; generally short chains; liquefying (gelatin); sometimes producing much gelatinous material (Leuconostoc).

2. Parasitic streptococci; generally in long chains.

(*a*) Type of Diplococcus pneumoniæ.

(*b*) Type of Streptococcus pyogenes.

B. *Merista:* Growth in two dimensions; division at right angles.

1. Tetragenus: Typical and fixed arrangement in tetrads.

2. Group of Diplococcus gonorrhœæ. Arrangement chiefly diplococcal. Does not stain by Gram's method.

3. Group of Staphylococcus pyogenes. Growth in second dimension often abolished; consequently, besides diplococci and tetrads, short chains also occur.

C. *Sarcina:* Growth in three dimensions in space.

II. Bacillaceæ: Bacilli.

1. Group of colorless sulphur-bacteria. Beggiatoa; Thiothrix. Usually large pseudo-threads; no spores; develop in medium containing H_2S.

2. Group of Leptothrices. Non-cultivatable inhabitants of water; form pseudo-threads; no spores.

3. Group of Cladothrices. For the most part non-cultivatable. Habitat, water. Form pseudo-threads with false branchings; spores; also present in artificial cultures of C. intricata.

4. Group of Hay bacilli. Large (mostly), easily cultivatable, saprophytic, sporulating bacilli.

5. Group of Anthrax bacilli. Distinguished from the foregoing chiefly by the mode of germination of spores.

6. Group of Malignant Œdema. Large, sporulating, anaerobic, pathogenic, and saprophytic bacilli.

7. Group of Symptomatic Anthrax (*Rauschbrand*) and butyric-acid bacilli. Large bacilli; saprophytic and parasitic; generally anaerobic; before sporulation show spindle-shaped swellings (Clostridium).

8. Group of Tetanus bacilli. Moderately large bacilli with end spores (*Köpfchensporen*). Mostly anaerobic saprophytes and parasites.

9. Group of Proteus: Non-sporulating bacilli; great variation in dimensions of bacilli and colonies. Gram stain mostly negative.

Addition: Liquefying bacilli, pathogenic for warm-blooded animals.

10. Group of fluorescent bacilli. Medium-sized, non-sporogenous bacilli; produce fluorescing pigment; all transitions from non-liquefying to rapidly liquefying kinds. Gram stain negative.

11. Groups of pigment bacilli.

12. Group of water bacilli; usual habitat, water; easily cultivated; medium-sized and small; non-sporogenous; all grades of liquefaction; Gram's method gives negative results.

Addition: Phosphorescent bacilli.

13. Group of Nitrobacteria.

14. Group of Aerogenes and Rhinoscleroma bacilli. Non-motile; of medium size, mostly plump, asporogenous, non-liquefying bacilli. Gram's method negative.

Addition: Bacteria of lactic acid, acetic acid, and ropy fermentations.

15. Group of Colon and Typhoid bacilli. Motile, medium-sized, generally delicate, non-liquefying and asporogenous bacilli. Gram's method gives negative results.

16. Group of Hemorrhagic Septicæmia. Moderately large or small, non-liquefying, asporogenous, highly pathogenic bacilli. Gram negative.

Addition: Bacteria of hemorrhagic septicæmia in man.

17. Group of Bacillus tenuis sputigenus. Bacilli of variable size; non-sporogenous and non-liquefying. Gram negative.

18. Group of Influenza bacillus: Minute asporogenous bacilli; mostly obligatory parasites; Gram negative.

19. Group of Hog Erysipelas (*Rothlauf*) bacilli. Minute asporogenous bacilli; growth on artificial cultures slight; Gram positive.

20. Group of Glanders and Pseudo-tuberculosis bacilli. Small bacilli; asporogenous; Gram negative.

21. Group of Diphtheria bacillus: Medium-sized and small bacilli; asporogenous; bizarre rods; Gram positive.

22. Group of Tubercle bacillus: Small asporogenous bacilli; growth slow; Gram positive.

III. Spirillaceæ: Spirals.

1. Group of Saprophytes; cultivated with difficulty or not at all.

2. Group of Saprophytes; cultivatable; non-liquefying.

3. Group of Saprophytes and Parasites; easily cultivated; liquefying.

4. Group of Obligatory parasites: S. recurrens.

While it is recognized that this system is inadequate and largely artificial, it yet fulfils the purposes of the bacteriologist in emphasizing those properties and functions which, at this time, render bacteriology an important subject of study to the chemist, agriculturalist, and physician. In spite of its defects it will therefore be employed with slight modification in the following chapters, but it will be drawn upon only to the extent required to cover the several groups of pathogenic bacteria of which we shall treat.

The Metabolic Products of Bacterial Growth.

The metabolism of bacteria can be understood in its entirety only after the entire list of chemical changes which they set up in process of their growth and multiplication and the mode of origin of the various chemical substances—evanescent and permanent—formed shall have been demonstrated. That the metabolism is complex has been proved by the number and composition of the chemical products already known. Gotschlich[7] enumerates the most common products found in cultures, the list containing: gases, such as CO_2, H_2, CH_4, H_2S, NH_3; nitrates, water, sulphur; volatile substances, trimethyl-

amine, alcohol, formic acid, acetic acid, propionic acid, butyric acid; oxy-acids and polybasic acids, lactic, malic, succinic, oxalic, and tartaric acids; sulpho-acids among which are taurin; amides such as leucin, alanin, etc.; aromatic bodies, tyrosin, phenol, cresol, hydroparacumaric acid; indol; pigments; carbohydrates; peptones; alkaloidal and albuminoid poisonous substances—ptomains and toxins; and hydrolytic ferments. The character of this metabolism will depend upon the nature of the organism and the composition of the medium upon which it is cultivated. Only the most prominent of the products of metabolism will be considered in this article.

Pigments.

More than one hundred known bacterial species produce pigments of various kinds. The formation of pigment, its color and nature, serve to distinguish bacterial species. While an important physiological function, this pigment formation is subject to variation. The amounts, intensity of color, and, indeed, the actual appearance of the pigment at all depend upon definite conditions of growth, such as the composition of the medium, the access or exclusion of oxygen, and, to a less degree, the influence of light.

Beyerinck has divided the chromogenic bacteria into three groups —chromophoric, chromoparic, and parachromophoric. In the group of chromophoric bacteria the pigment plays an important part in the life history of the cell. It constitutes an integral part of the cell and is united to it in a manner analogous to the union between chlorophyll and the chromatophores of the higher plants and hæmoglobin in the red blood corpuscles. In this group Beyerinck includes the red sulphur bacteria and the green, red, yellow, and brown varieties which do not liquefy gelatin. The chromophoric or true pigment bacteria give off the pigment either as such or, in a colorless form, as a secretion which diffuses into the surrounding medium. As belonging to this group are reckoned the Bacillus prodigiosus, cyaneofuscus, pyocyaneus, syncyanea, and other fluorescent varieties. The parachromophoric bacteria also excrete pigment, but the latter, instead of becoming admixed with the medium in which they grow, remains attached to the bacterial bodies. Examples of this group are found in B. violaceus and B. janthinus.

Migula does not agree entirely with the views of Beyerinck, but states that only in the red sulphur bacteria and the green varieties of pigment bacteria is the pigment to be regarded as forming an integral part of the cell, since in other instances it is largely, if not entirely, thrown off into the surrounding substance. Moreover, he finds great difficulty in convincing himself that in the parachromophoric bacteria

the pigment is really upon the cells rather than in their immediate neighborhood. With our present optical means this distinction is very difficult if not impossible.

Pigment bacteria owe their peculiar colors sometimes to one pigment and sometimes to several which they produce. More than one kind of pigment are found in B. pyocyaneus, syncyanea, aurea, erythrogenes, and erythromyxa.

The pigments exhibit different chemical properties. Indeed, the diversity in their solubilities is sufficiently striking to have led Migula to use this fact as a basis of classification. He distinguishes the pigments according as they are soluble (1) in water, (2) in alcohol, being at the same time insoluble in water, and (3) in neither water nor alcohol.

1. *Pigments soluble in water.* To this class belong all the fluorescing pigments and all such pigments as give to gelatin a red, brown, or black hue. Certain yellow pigments which fail to diffuse in gelatin are included here because of their solubility in water.

According to Thumm, all fluorescent pigments are identical; the different appearances met with in cultures from various sources probably depend upon differences in intensity of color and composition of the culture medium. The isolated pigment is a yellow mass, soluble in water, but insoluble in alcohol, carbon disulphide, ether, and benzene. Watery solutions are orange-yellow or yellow depending upon their concentration. The fluorescence is blue; the addition of an alkali alters it to green. Acid media destroy the fluorescence without suppressing the formation of the pigment. Chemically the body appears to be an albumin.

Similar fluorescent pigments are formed by the Bacillus pyocyaneus and syncyanea; these bacilli produce, however, additional pigments. The second pigment found in cultures of B. syncyanea is of a steel-blue color, and is produced more especially in acid media.

Several different pigments have been obtained from growths of B. pyocyaneus. In 1859 Fordos isolated a pigment from blue pus which he called *pyocyanin*. Gessard later obtained the same body from cultures of the organism. From it Gessard caused to split off by oxidation a second brownish-red pigment to which he gave the name of *pyoxanthin*. Ernst distinguishes two varieties of B. pyocyaneus and believes that they form different pigments. From his β-variety, by agitation with chloroform and evaporation, he obtained azure-blue crystals, while the α-variety gave, after the same treatment, greenish-shining crystals. Ledderhose obtained pyocyanin from cultures by extracting them with chloroform and succeeded in combining it into a picrate. The empirical formula, as determined by him,

is $C_{14}H_{14}N_2O$. Ledderhose believed that the pigment was secreted as a leuco-product which required atmospheric oxygen to convert it into pigment. Kunz, Babes, and Gessard found a third pigment. We may distinguish the following pigments as being elaborated by the Bacillus pyocyaneus: (1) Pyocyanin, (2) a fluorescent pigment, and (3) pyoxanthin. Jordan distinguishes two primary pigments: (*a*) Fluorescigenic, (*b*) pyocyanigenic. A third black pigment may be obtained from the latter by oxidation. Rarely the bacilli possess pyocyanigenic properties only.

A series of pigments soluble in water, which have been insufficiently investigated, are formed by B. lactis erythrogenes, B. microspira nigricans, and some others.

2. *Pigments insoluble in water, but soluble in alcohol.* The great majority of bacterial pigments belong to this class. The most important are the lipochromes. These are distinguished by means of the lipocyanin reaction of Zopf. Treated with concentrated sulphuric acid, the red or yellow mass becomes converted into blue granules or crystals. Migula suggests that bacteriopurpurin and the green pigments of certain bacteria may be related to the lipochromes. This pigment is widely disseminated. A second kind of pigment is formed by B. prodigiosus, B. ruber, B. kiliensis, etc. While showing in some respects a resemblance to lipochrome they fail to give the lipocyanin reaction. The pigment of B. violaceus, soluble in alcohol, is insoluble in chloroform. Whether the pigment produced by B. janthinus is identical with that of B. violaceus requires further study to determine.

3. *Pigments insoluble both in water and alcohol.* Very few of the known chromogenic bacteria yield pigments belonging to this class. Migula mentions only two species; the Micrococcus cereus flavus and B. berolinensis. The yellow pigment of the former is soluble in boiling ten-per-cent. hydrate of potassium; while that of the latter is soluble in hydrochloric acid. Their chemistry is not understood.

Conditions favoring or hindering pigment production. Some chromogenic bacteria produce pigment under all conditions of growth, while others, when subjected to the influence of certain external conditions, show modifications in their pigment production. It is possible to suppress by artificial means the chromogenic capacity; but it is not always possible to call it forth when once lost. In respect to this property there is great variation in different organisms. While the lipochromogenic bacteria rarely and with great difficulty lose this power, the fluorescent bacteria are not so stubborn, the least resistant forms being some species belonging to Group 2, among which are B. prodigiosus, B. kiliensis, and others.

For the production of pigment a plentiful supply of oxygen is of the utmost importance. Very few bacteria are known which develop pigment only when oxygen is excluded. The best known example of the latter is the Spirillum rubrum of Esmarch; a second is the Diplococcus pyogenes of Pasquale, and a third, B. rubellus of Ogata. In all these the probable explanation of the loss of color, when air is permitted access, is the ease with which the pigments are oxidized to leuco-compounds.

Temperature always plays an important part. B. prodigiosus, for example, while growing abundantly at the temperature of the body, fails to form pigment. Indeed Schottelius has shown that by successive cultivation at this temperature colorless races may be produced which fail to form pigment, even when grown at the most appropriate temperatures. A similar behavior has been noted in B. kiliensis, B. ruber, B. indicus, and other violet and blue chromogens. In some cases the pigment production is not entirely inhibited, but is only weakened by this temperature. The Staphylococcus pyogenes aureus has its chromogenic activity diminished by temperatures of 37° to 38° C. Finally, the lipochromic varieties are not influenced by the foregoing temperatures.

Light, inasmuch as it does not act injuriously upon the growth of the bacteria, has only a slight effect on chromogenesis. The varieties especially affected are the green chromogens, the red sulphur bacteria, and the Micrococcus ochroleucus (Prove) which produce pigment only in the light. The bacteria which contain chromophyll and the red sulphur bacteria, according to the investigations of Engelmann, are capable, under the influence of light, of eliminating oxygen.

The composition of the medium is of great importance. For the fluorescent varieties Gessard showed that the presence of phosphoric acid was essential to pigment production. Pyocyanin is, however, formed in its absence (Jordan). Thumm has made interesting observations upon the influence of chemical agents. Chloride of calcium is superfluous in media, provided that magnesium sulphate be present. Should the latter be wanting the presence of calcium chloride, which otherwise is of no value in promoting growth, causes a rich development of the bacteria, but no pigment is formed. Potassium phosphate is necessary, for both its potassium and phosphoric acid constitutents. Salts of organic acids also exhibit an unequal value. For example, the bacillus of blue milk when grown in a medium containing ammonium citrate produces only the blue-black pigment; in alkaline asparagin solution only the fluorescent; while in the presence of tartrate and lactate of ammonium both pigments are formed.

Similar diversities in the intensity of pigmentation noted in a given organism, when cultivated upon agar-agar, gelatin, potato, and other media, are matters of common observation. The favorable influence of carbohydrates is shown in the intense pigmentation of B. prodigiosus, B. kiliensis, B. violaceus, and Staphylococcus aureus when grown upon boiled potato.

Chromogenic power is often lost in artificial cultures. The causes of this alteration in function are probably associated with the reaction of the media and the influence of the metabolic products excreted by the organisms themselves. That unfavorable conditions of growth may bring about this loss has already been stated. Varieties of bacteria, colorless upon certain media, may suddenly develop color on others, as is seen in the transplantation to potato from agar-agar of colorless colonies of Staphylococcus aureus. Gessard found no difficulty in obtaining colorless races of B. pyocyaneus by employing high temperatures and media of particular composition. The longer the colorless races are cultivated, the more difficult are they to bring to pigment production again. For the later purpose Migula recommends as media giving the best results potato and a medium made of rice.

Ferments.*

Bacteria along with other microorganisms are capable of bringing about those transformations of organic substances to which the name of fermentation is applied. These results are effected through the action of (1) the living bacterial cell, and (2) separable ferments or enzymes, whose activities are comparable with those of ptyalin, trypsin, and pepsin as obtained from animal secretions. Whether in the last instance all the fermentation is due to the action of enzymes and the living cell is indispensable only because it elaborates such ferments, cannot with our present knowledge be certainly affirmed. It is, however, probable that complex changes such as, for example, the decomposition of sugar into alcohol and carbon dioxide may be brought about by such soluble ferments obtained by expression from the yeast cell (E. Buchner). The enzymes elaborated by bacteria are capable of converting albumin into peptone, starch and cellulose into sugar and, perhaps, sugar into alcohol and carbon dioxide. Others are capable of splitting up fats and of transforming saccharose into fermentable glucose and fructose.

1. *Diastatic ferment.* Beyerinck, following the lead of certain of the French writers, has proposed to call this class *amylases.* These

*The classification of Gotschlich has been followed in this chapter, and his treatise[7] has been largely drawn upon in its preparation.

ferments have in common the property of converting starch into sugar. They are widely diffused in nature. In animals they constitute the ptyalin of the salivary secretion and the diastatic ferment of the pancreatic secretion, the glycogenic ferment of the liver, and a similar ferment in urine (Selmi, Béchamp, and Baltus) and in the blood (Bial, Röhmann). In plants they are found in germinating seeds, especially those of the Gramineæ. Diastatic ferments have been found not infrequently in microorganisms, as by Marcano in bacteria present in the outer envelope of corn. Hüppe demonstrated this property in B. acidi lactici and Fermi found it in a large number of bacteria. Fermi isolated the ferment by precipitation with alcohol from cultures of B. anthracis, B. subtilis, and B. megatherium, and from Vibrio choleræ asiaticæ, Finkler-Prior, tyrogenum.

2. *Inverting ferments.* The inverting ferments convert the disaccharides (saccharose, maltose) into simple hexoses. The best known is the invertin which transforms saccharose into d-glucose and d-fructose. These ferments are present in the digestive tract of animals, but thus far have not been demonstrated in the higher plants. On the other hand they are elaborated by the moulds, yeasts, and bacteria. Gayon found an inverting ferment in penicillium and aspergillus. Yeasts elaborate it, although probably not every species of blastomycetes is capable of doing so. Roux and Hansen have described yeasts which fail to invert saccharose. As regards the production of invertin by bacteria, Gayon described it in cultures of B. anthracis, an observation which was not confirmed by Fermi and Montesano. Sclavo found an inconstant inverting action in cultures of Vibrio Metchnikovii and V. choleræ asiaticæ. Fermi and Montesano tested seventy different kinds of bacteria and found an inverting ferment constantly only in B. megatherium, B. kiliensis, B. fluorescens liquefaciens, and Proteus vulgaris; and inconstantly in Vibrio choleræ asiaticæ and V. Metchnikovii. The variable action of B. coli communis upon saccharose doubtless depends upon variations in inverting capacity.

3. *Glucosidic ferments.* These ferments act upon glucosides and upon certain sugars (fructose and galactose). They decompose glucosides, which, taking up a molecule of water, are converted into their original components, d-glucose and another inconstant body—depending upon the particular glucoside being decomposed. In the decomposition of the glucoside amygdalin, glucose, benzaldehyde, and hydrocyanic acid are formed. The change is effected under ordinary conditions through the influence of the ferment emulsin which occurs in nature with the glucoside. Emulsin was found by Bourquelot in Aspergillus niger, by Girard in Penicillium glaucum, and by Fermi

and Montesano in very few (only three) species of bacteria. The decomposition of amygdalin by microbes seems to be caused by the living protoplasm rather than by separable ferments.

4. *Peptonizing ferments.* The corresponding ferments to the peptic and pancreatic (trypsin) secretions belong to this class. The ferments are represented in the higher plants by the enzyme contained in carica papaya (papain). This ferment and the bacterial peptonizing examples agree with trypsin in acting in an alkaline medium. These enzymes are widely present among certain bacteria and upon them depends the common liquefaction of gelatin by microbic growth. Certain bacteria are also capable of peptonizing fibrin.

The first demonstration that peptonization may occur independently of the bacteria themselves was made by Bitter, who first killed the cholera vibrio in cultures by careful heating and found that the fluid still possessed energetic peptonizing properties. Rietsch and Sternberg demonstrated in cultures of liquefying bacteria—V. choleræ, Finkler-Prior, B. prodigiosus, pyocyaneus, Staphylococcus aureus —peptonizing ferments; while in cultures of non-liquefying bacteria, as B. typhosus and B. tuberculosis, they failed to find them. Fermi showed these ferments to be elaborated by a large number of microorganisms, and he obtained by precipitation active enzymes in the dried state. The most active ferment was obtained from the Vibrio Finkler-Prior.

These ferments are non-dialyzable. They resist higher temperatures when in the dry state much as does trypsin; in watery solution they are much more sensitive. The ferment of V. Finkler-Prior was still active after dry-heating for ten minutes at temperatures of 120°–140° C. Warming in solution to 70° C. destroyed its action. Other bacterial ferments are less resistant, that of B. prodigiosus being killed at 55° C. Acids are injurious, while the bacterial ferments are very resistant to alkalies. The moulds form peptonizing ferments capable, like pepsin, of acting only in an acid (HCl) medium. Gelatin is more readily peptonized than fibrin; some bacterial ferments, indeed, are incapable of digesting fibrin. Ferments are even more resistant to chemical disinfectants (carbolic acid, sublimate) than are spores. The peptonizing ferments differ among themselves and, of course, from other kinds of ferments. Some bacteria produce more than one kind at the same time. MacCallum and Hastings[5] have described a pathogenic micrococcus which makes the lab-ferment and a peptonizing ferment simultaneously.

For the production of ferments Fermi regards albuminous culture media as necessary, while Liborius looks upon free access of oxygen as essential. In a medium free from albumin Fermi found that B.

subtilis was capable only of forming a peptonizing ferment. Agents which inhibit bacterial growth (carbolic and salicylic acids) also diminish the fermentative activity, probably because of their influence upon the growth. However, certain chemicals (quinine, antipyrin, strychnine), which do not inhibit growth entirely, prevent the production of ferment. This latter is therefore no necessary function of bacterial activity. Delbrück has described a peptonizing ferment—peptose—in yeast cells.

5. *Lab-ferments.* Like rennet, these ferments bring about the coagulation of casein. Duclaux[8] and Hüppe first drew attention to their occurrence in bacteria. The precipitation occurs in faintly acid, amphoteric, and alkaline milk. After precipitation the coagula are often peptonized by a tryptic ferment. The action of B. pyocyaneus, B. anthracis, and Micrococcus zymogenes (MacCallum and Hastings) is of this dual nature. Not all coagulation of milk by bacteria is caused by ferments; some bacteria bring about this result by acid production alone. Cohn isolated the lab-ferment from the bacterial cultures, and separated it from the associated tryptic enzyme; the former is destroyed by temperatures varying from 63°–75° C. MacCallum and Hastings found the filtered cultures to contain both ferments, and that chloroform did not interfere with their activity. The quantity of lab-ferment formed is subject to variation according to the species, age of culture, and temperature during growth. In opposition to what happens in the case of the proteolytic ferment, more lab-ferment is produced at 20° than at 37° C. Where both ferments are formed simultaneously the presence of the lab-ferment may be masked by the complete peptonization of the casein.

6. *Cellulose-dissolving ferments.* These are believed to be produced by several varieties of B. butyricus and V. rugula.

Urine-ferments have the property of splitting up certain amide compounds, such as urea, which is converted into ammonium carbonate, and hippuric acid, and into glycocoll and benzoic acid. This transformation of urea was formerly ascribed to the micrococcus ureæ, and Musculus isolated from the ropy urine a urea-splitting ferment which he thought was produced by this organism. Miguel has described some sixty varieties of bacteria—cocci and bacilli—which are capable of splitting up urea. These exist in the air, and in fresh but still more often in stagnant water. The enzyme which he isolated from the bacteria he called urasin. It is quickly oxidized by atmospheric oxygen.

7. *Fat-splitting ferments.* Ferments capable of splitting up neutral fats into glycerin and fatty acids have been described by Sommaruga, who observed this action in a number of microorganisms

which apparently utilized the glycerin for their growth and multiplication. It remains to be proved whether this action is due to an insoluble enzyme or is not rather the direct result of the action of the living cellular protoplasm.

Ptomains, Toxins, Toxalbumins, and Antitoxins.

1. *Ptomains and toxins.* Poisonous products have, at different times and by different investigators, been isolated from substances in which bacteria have developed. The first of these were obtained from putrefying substances containing mixtures of various bacteria. Of this nature was the *sepsin* of Bergmann and Schmiedeberg. Zuelzer and Sonnenschein, Hager, Otto, Bence-Jones, Dupré and Selmi, and others extracted poisonous substances from bacterial growths which in their action resembled various poisonous alkaloids, such as coniine, atropine, curarine, morphine, etc., with which vegetable poisons they compared these substances. Selmi regarded these bodies, to which he applied the term *ptomains* (cadaveric albuminoids), as organic bases produced during the putrefaction of animal and also of certain vegetable substances. The first of these bodies to be obtained in a pure state was collidin, which Nencki obtained by digesting gelatin with pancreas. Brieger, his pupil, isolated from putrefying substances a number of pure crystalline nitrogen-containing bases, some of which were without toxic properties, whereas others were highly poisonous. To this last group Brieger applied the special name of *toxin.* In the group of non-poisonous cadaveric albuminoids, or such as are poisonous only when given in very large quantities, are to be included neuridin, gadinin, putrescin, cadaverin, saprin, cholin, mydatoxin, and mydin. A considerable number of these bases are toxic even in very small doses (toxins). Among these may be mentioned peptotoxin, neurin, muscarin obtained from decomposing fish and identical with the toxic principle of poisonous mushrooms, methylguanidin, mytilotoxin, tyrotoxicon—the last body having been obtained by Vaughan from decomposed milk and ice cream. Gautier showed that in the normal metabolism of the animal body substances of an alkaloidal nature, to which he gave the name of *leucomains,* were produced. With these he classes the extractive substances, such as xanthin, creatinin, and others. The studies of Nencki, Brieger, and Gautier, while throwing much light upon the products of putrefaction and even upon normal animal metabolism, did not indicate the character of the substances produced by bacteria of one kind so much as the products resulting from mixtures of these organisms. The study of pure cultures of a number of mi-

croorganisms indicated that in these also similar bases could be found. Thus Brieger obtained certain ptomains from pure cultures of various bacteria. From the typhoid bacillus he isolated typhotoxin, from the cholera vibrio cadaverin along with two other bodies present in very minute quantities, while Kunz demonstrated spermin in cholera cultures. Cadaverin was also obtained from the cultures of Vibrio Finkler-Prior; cultures of the tetanus bacillus yielded at least four toxins. Hoffa and Heim and Geyger obtained similar bodies from cultures of the anthrax bacillus, and Novy isolated the so-called susotoxin from growths of the swine-plague organism. From cultures of the hog-cholera bacillus de Schweinitz obtained, besides cadaverin and a primary amin, a ptomain which was obtained as a platinum salt that exhibited slight toxic action. Leber isolated from cultures of Staphylococcus pyogenes aureus the crystalline alkaloid to which he gave the name of phlogosin; and Levy obtained, from fluidified gelatin cultures of Proteus Hauseri, a poison which he identified with the sepsin of von Bergmann and Schmiedeberg.

2. *Toxalbumins, toxoids, and antitoxins.* The absence of these crystalline bodies from cultures of some of the most exquisitely pathogenic bacteria, and the want of correspondence between the symptoms caused by these substances on the one hand and the specific microorganisms on the other, led some to the belief that the toxic ptomains were not the specific factors in bacterial action. The separation by Roux and Yersin from cultures of B. diphtheriæ of a poisonous body belonging to the class of albumins, in which no crystalline substance could be detected, and the discovery that this substance was capable of reproducing the essential clinical and pathological phenomena of the disease diphtheria, opened the way for the study of the class of toxins to which the name *toxalbumins* has been given. This group of toxic substances, it is now known, has representatives among the constituents of the higher plants and animals (castor oil, jequirity, and croton-oil beans, snake-venom), as well as those of the microscopic world, in the latter being formed by B. diphtheriæ, B. tetani, and botulismus, and probably by still other organisms. These toxalbumins are sensitive in watery solutions to higher temperatures. At 60° C. after a short exposure, they are destroyed and the boiling temperature renders them immediately inactive. According to Buchner, the presence of neutral salts, as in the blood, renders them somewhat more resistant to higher temperatures. They are soluble in water, are precipitated by alcohol and neutral salts; they are with difficulty forced through porcelain filters; they are non-dialyzable, and they give the well-known chemical reactions for albumins.

As to the ultimate chemical nature of these bodies we have very

little definite knowledge. Some hold that they are unorganized ferments or enzymes, while other writers have endeavored to prove that they are of a non-albuminous nature. Roux and Yersin, and Sidney Martin have favored the view that the diphtheria toxin is an enzyme; and Stillmark holds that the same is probably true for the toxalbumin, ricin (from ricinus communis). Nencki, going much further, would include among these the alexins or defensive proteids of blood serum. Tizzoni and Cattani regard the tetanus antitoxin as of the nature of an enzyme. Pfeiffer has recently expressed the same opinion with reference to the "anti-body" which causes rapid disintegration of cholera bacilli in intraperitoneal inoculations in guinea-pigs. An important contribution bearing upon the enzyme theory of the toxalbumins has just been supplied by the observations of Ehrlich and Morgenroth [10] upon the action of lysin. Bordet had pointed out the analogy between Pfeiffer's reaction (see page 541) and the phenomenon observed by himself that the blood of guinea-pigs, repeatedly injected with defibrinated rabbit's blood, exerted a solvent action on the blood corpuscles of rabbits *in vitro*, the solution being preceded by agglutination of the erythrocytes. Warming for half an hour to 65° C. of the blood serum of the guinea-pig possessing this action destroys the hæmolytic function, but the agglutinating property remains unaffected. The serum rendered inactive by heating regains its hæmolytic property on the addition of serum from the normal guinea-pig or even the rabbit. Ehrlich and Morgenroth have observed that a similar effect is produced in the blood of the goat when that animal has been treated by subcutaneous injections of sheep's serum. The serum of the goat rapidly dissolved the erythrocytes of sheep's blood *in vitro*, although no agglutinating property could be made out. According to these authors it is necessary to assume for hæmolysis, as Pfeiffer has done for bacteriolysis, the existence of two substances, one specifically active, the *immunizing substance*, and another less resistant and non-specific, to which the name of *addiment* is applied. The immunizing body possesses a specific haptophorous group by which it is fixed to the red corpuscle. This latter has not the power to take up addiment, while the immunizing body is capable of combining with it. After the corpuscle and the immunizing body are united, the addiment is capable of fixation and the solution of the corpuscles begins, after the manner of the action of a digestive ferment. In explaining the solution of bacteria or of red corpuscles, according to the phenomena observed, it is assumed that the immunizing substance combines in the blood with a small amount of normally present ferment (addiment) and transmits this to the bacteria or red corpuscles.

Pfeiffer and Proskauer have proved that the body which causes the disintegration of cholera spirilla when treated according to Pfeiffer's method, does not belong to the albumins, in the strict sense of the word, in that it resists for a time the digestive action of both pepsin and trypsin, and is capable through dialysis of being separated from demonstrable portions of albumin, albumoses, peptones, and salts. It appears also not to belong to the nuclein bodies. Its analogies are chiefly with the group of ferments. If, however, it is a ferment, it must be regarded as one of a very peculiar nature in that it acts upon only one kind of bacterial protoplasm, causing it to undergo swelling, disintegration, and solution just as albumin and fibrin do when acted on by pepsin and trypsin. It is comparable, on account of its action upon one kind of bacterial protoplasm only, with the ferments of yeast cells which, as pointed out by Emil Fischer, are capable of splitting up only sugars of definite chemical constitution, while upon all others they are without action. There is, then, strong theoretical support for the enzyme-like nature, not only of the toxins, but of the anti-bodies (Pfeiffer, Grubler, Durham, etc.), and perhaps also of the antitoxins whose action would be of the nature of a fermentative destruction of the poison molecules.

Dissent from this view has, however, not been wanting. Brieger and Fraenkel oppose it so far as regards the diphtheria toxin; and Fermi considers as militating against it the fact that of one hundred and fourteen described pathogenic and toxin-producing microorganisms only twenty-six yield proteolytic ferments; while, on the other hand, of one hundred and thirty-four known enzyme-producing bacteria only twenty-five are pathogenic, that is to say, produce toxalbumins. The investigations of Brieger and Cohn, and of Brieger and Boer have, more than any others, shaken the belief in the albuminous character of these bodies. The first two investigators succeeded in separating the tetanus poison almost entirely from admixture with albuminous substances. The poison isolated failed to respond to Millon's reagent, or the xanthoproteic test for albumin, although still giving a faint biuret reaction. Brieger and Boer obtained from bacteria-free cultures of B. tetani and B. diphtheriæ, insoluble zinc compounds which contained the whole of the toxin equivalents and which were absolutely free from albuminous peptones.

Not all bacteria yield their poisons to the surrounding medium in which they grow. Pfeiffer found that the cholera bacilli produced a poison which adhered to their bodies and probably formed an integral part of their structure. The dead cultures possess marked poisonous properties. The allied vibrios, namely, V. Metchnikovii and the comma bacillus of Finkler-Prior, elaborate a closely related

poison, while the virus of the typhoid bacillus is contained similarly within the bacterial cell, and sterilized cultures of the microorganisms are still toxic. The toxic proteids contained within the bacterial cells in these instances are doubtless different from the bacterio-proteid of Buchner in which specific properties are lacking; they probably also differ from the pyretogenous agent, pyrotoxin, isolated by Centanni from saprophytic and pathogenic bacteria, to which, in his opinion, the fever of infectious diseases is due.

The poisonous substances attached to the protoplasm of bacteria —cholera bacilli, typhoid bacilli, gonococci—have been denominated toxins. Their relationship to the toxins, in the proper sense of the term, is thus far unknown, in that their study has been beset with great difficulties. The poisons are firmly adherent to the cell and often become free only after disintegration of the latter. In the animal body they seem to find especially favorable conditions for their solution, if we assume that their activity depends upon such a liberation of the poisons. Morgenroth has suggested that the action *intra vitam* may take place without separation from the cell in a manner already demonstrated for certain of the vegetable ferments (*e.g.*, monilia candida).

A characteristic of all toxins is the capacity to induce the formation of *antitoxins*, according to Behring's law, which holds that the introduction of such toxins in suitable doses is followed by the evolution of their specific antitoxins; and, further, that by repeated and continued inoculations this active immunity can be brought to a high degree of perfection. This capacity of the bacterial toxins to produce antitoxins is shared by their related vegetable poisons (phytalbumoses), ricin, abrin, crotin, and by snake-venom.

A second characteristic physiological peculiarity of the toxins is found in the period of incubation which precedes the manifestations of their action. From the time of the absorption of the poison until the appearance of anatomical or clinical phenomena a number of hours or even days elapse. This incubation period cannot, except in the case of snake-venom, be eliminated by increasing the dose of the poison.

Very recently Ehrlich has made important contributions to our knowledge of the nature of toxins and antitoxins. The toxins produced by bacteria form a minimal portion only of the contents of the culture tube in which the bacteria have grown. These media may be regarded simply as vehicles in which the toxins and other substances are dissolved. These toxic solutions are subject to changes owing to which their poisonous properties may be lost, such alterations arising from decomposition of the toxins.

The mode of origin of ptomains, toxins, and toxalbumins has been variously regarded. The two main possibilities are: (1) They may represent decomposition products of the albuminoid constituents of the culture-media as the result of the action of the bacteria; or (2) they are or have been integral portions of the bacterial cells or some immediate derivative from them. Still another view has been brought forward by Sidney Martin, who has suggested that the diphtheria toxin is produced in the animal body through the action of an enzyme contained within the bacterial growth.

A solution of the question whether the toxins arise by decomposition of the cultural albumin or within the protoplasm of the bacteria has been arrived at through the use of artificial culture media free from proteid (*e.g.*, Uschinsky's fluid). The ptomains are formed from the cultural albumins and are more abundant when growth has taken place under anaerobic conditions. On the other hand, certain toxalbumins—from cultures of B. diphtheriæ, B. tuberculosis, B. mallei, and B. tetani—originate within the cell protoplasm or are close derivatives of such intracellular bodies.

Ehrlich has found that besides the diphtheria toxin, a second toxic substance which possesses modified poisonous properties, *toxoid*, exists in cultures of B. diphtheriæ. The presence of this body as well as of the toxin is demonstrable only by animal experiment.

Concerning the *relation of toxin to antitoxin* there are two opposite views. According to the conception of Behring and Ehrlich, the neutralization of the toxin is of the nature of a chemical reaction in which toxin and antitoxin unite in equivalent proportions, the resulting compound being free from poisonous properties.

On the other hand, Buchner, Roux, Metchnikoff, and others hold that the somatic cells play an important part, in that they receive a stimulus from the antitoxin and are thus enabled to destroy the toxin. Ehrlich has, however, shown that neutralization takes place *in vitro* as well as within the animal body. This has been shown for ricin (Ehrlich), the poison of the blood of the eel and its antitoxin (Kossel, Gley, and Camus), crotin (Morgenroth), and snake-poison (Stephens and Meyers). A similar chemical union takes place in the animal body. Knorr has shown that the tetanus toxin, when introduced into the circulation, disappears from the blood long before there is any manifestation of tetanic symptoms. Close observation has shown that the time required for this elimination from the blood is different for different animals. In the rabbit, for example, the disappearance is more rapid than in the more sensitive guinea-pig. Ehrlich assumed that in a given case the chemical union of the toxin with protoplasm was the precursor of the appearance of symptoms of the

disease; and in that the tetanus poison seemed to exert its chief action upon the central nervous system, he assumed that the combination occurred in this situation. Wassermann confirmed these theoretical considerations by bringing the toxin into contact with emulsions of brain and spinal cord and observing their union into non-poisonous compounds. Still other organs, as, for example, the liver and kidneys, are capable, as has been shown by Dönitz, Roux, and Borrell, of fixing the tetanus toxin.

This fixation of the toxin depends, according to Ehrlich's theory, upon a definite structure of the protoplasmic molecule. He holds that every functioning protoplasmic mass consists of a nucleus and its lateral chains (*Seitenketten*) which subserve a definite function; and that the possibility of fixing toxins depends upon the existence of a specific chemical relationship in the lateral chains, the necessary condition to this union being a definite configuration of both molecules—protoplasmic and toxic. The union of the toxins with the lateral chains causes the latter to be thrown out of function, with the inevitable result that a physiological defect is produced. Whether, now, this defect is to be permanent or transient depends upon the degree of regenerative power in the protoplasm—a power which is among its natural functions. The regeneration, as is the case with pathological regeneration in general (Weigert), is not an exact equivalent, but usually an over-compensation. If, therefore, the toxin is introduced successfully, that is, in proper amounts at proper intervals, the regeneration of the lateral chains is gradually increased until a much greater number is produced than can be used by the cell or combined in its protoplasm. As a natural result, then, these superabundant lateral chains are cast off and find their way into the circulation, where they still preserve their natural affinity for toxin and constitute what is denominated *antitoxin*. "Every antitoxin possesses, therefore, a physiological analogue and the production of antitoxin is merely the accumulation of a physiological process of regeneration. The antitoxins are the necessary corollaries of the effects of the toxin; and in the light of this theory these bodies lose completely their mystical character of teleological mechanisms" (Ehrlich).

Toxoids, according to Ehrlich, have the general configuration of the toxin molecule; they are capable, therefore, of combining with the molecule contained within the lateral chains, but at the same time are so modified atomically as to be poisonous in a less degree than the toxins or, perhaps, have lost their toxicity entirely. These toxoids constitute an important part of the culture fluids and are formed during the spontaneous loss of toxicity of toxin solutions.

The presence of toxoids can be demonstrated in the study of the numerical relations of diphtheria toxin and its antitoxin. Toxoids whose combining capacity is less than that of the toxins are called by Ehrlich *epitoxoids*. These also occur in solutions of diphtheria toxin. That the toxoids are metamorphosed toxins is shown by the result of the treatment of tetanus toxin with carbon bisulphide, through which the toxicity is almost completely lost, while the immunizing power and capacity to fix antitoxin are still retained. The non-poisonous modifications (toxoids) possess, therefore, the specifically combining groups of the toxins. Ehrlich has shown that the transformation of toxin into toxoid proceeds in numerical proportion; either three toxin molecules are converted into two toxoid molecules, or the toxin molecule splits up into two equal portions—one being toxin, the other toxoid. The toxoids formed in this manner have not the characters of epitoxoid; they have either the same relation (syntoxoid) or they have an even closer relation (protoxoid) to the antitoxin than the toxin.

The exact conditions involved in the production of the toxoids are still to a large extent unknown. It is probable that the artificial weakening of toxins, often employed for purposes of immunization, through the use of chemicals or other methods (iodine, Roux; trichloride of iodine, Behring; heating, C. Fraenkel) may be effective in that such toxoids are produced which, while still possessing the power of generating antitoxins, have partially lost their poisonous properties.

The Technique of Bacteriology.

Culture Media.

General Considerations of Nutrition and Cultures.

Bacteria, like other living organisms, consist chiefly of water, containing as they do in round numbers eighty-five per cent. of this substance. This high percentage can be explained by the fact that they are water rather than land organisms. Analyses of bacteria freed as far as possible from admixture with culture media give the following results:

	Putrefactive bacteria (Nencki).	B. prodigiosus (Kappes).
Water	83.42	85.45
Albuminoids	13.96	10.33
Fat	1.00	0.70
Ash	0.78	1.75
Residue (not further analyzed)	0.84	1.57

The analyses are only broadly correct. Alterations in the composition of the media in which they are cultivated, as well as in the specific products of the germs, would probably introduce new factors. Nencki called the separated albuminoid *mycoprotein;* in the purified state it consists of carbon (52.39 per cent.), hydrogen (7.55 per cent.), nitrogen (14.75 per cent.), and oxygen (about 25 per cent.). It is probable that the toxins are closely related to the proteid of the bacterial protoplasm (A. Fischer). The granulose reaction (with iodine) given by the butyric-acid bacillus and certain bacteria found in the mouth, cannot be said definitely to prove the presence of a carbohydrate. The gelatinous material present in growths of leuconostoc and other slime-producing bacteria probably consists of a carbohydrate, dextran (A. Fischer). Special inclusions, such as sulphur, iron, and pigment, may still further modify the results of the analyses.

Although bacteria, like other organisms, require mineral and organic constituents for building up their bodies, the amount of mineral matter in their composition is so small that minimal additions of salts to culture media are sufficient. In artificial fluids the quantity, as a general rule, need not exceed 0.1–0.2 per cent.; for certain bacteria, however, especially the pathogenic forms, a proportion of 0.5 per cent. gives better results. For the regeneration of the mineral constituents, phosphoric acid seems to be the most essential, while, according to Proskauer and Beck, the chlorides may be dispensed with. Loew says that calcium salts are not necessary, and Kappes holds that calcium and magnesium may be substituted for by potassium and sodium. Iron is necessary to the growth of certain filamentous bacteria (iron bacteria); and sulphur, which is at one time derived from sulphates and at another time from organic sulphur compounds, is required by the sulphur bacteria. One group of sulphur bacteria can flourish only in the presence of hydrogen sulphide and from this gas they derive the sulphur essential to their growth (Winogradsky).

Carbon is derived from albumins, peptone, sugar, glycerin, fats, and certain organic acids (acetic, tartaric, citric). Alcohols may also supply it and ethylic alcohol is the favorite medium for the acetic-acid bacillus which continues to flourish in mixtures containing ten per cent. of this ingredient. Many other bodies—amido acids, ketones, etc.—can also be employed if properly diluted. Loew has described a bacillus which is capable of obtaining carbon from formates, and the nitrobacteria of Winogradsky utilize the combined and atmospheric CO_2 to the same end.

Nitrogen is obtained most readily from diffusible albuminoids;

ammonium compounds are less useful. Many bacteria, including certain pathogenic varieties, may be successfully cultivated in media free from albuminoids (Uschinsky's fluid, see p. 590). Naegeli was of the opinion that the nitrogen was obtained from the nitrates through processes of reduction in which nitrites and ammonia are produced. Such reduction, indeed, takes place; but that it is the source of the nitrogen in all cases is at least doubtful. A special property of the bacteria contained within the nodes upon the roots of the leguminosæ and allied plants should be mentioned. These organisms are capable of fixing the atmospheric nitrogen which is thus rendered available for use by the higher plants with which they live in symbiotic relation. These bacteria are also able to utilize nitrogen combined in ammonia, in the presence of which they fail to fix the free element of the atmosphere (Winogradsky). Great variations are found in the capacity of the various pathogenic bacteria in obtaining nitrogen from substances containing this element in different combinations. While the anthrax bacillus is almost exclusively dependent upon peptone for its nitrogen, the pyocyaneus bacillus can utilize that element when it meets with it in combinations of nitrates. Many more can extract it from ammonia (B. coli; B. subtilis; V. choleræ asiaticæ); a few, among them B. typhi, can utilize amido compounds (asparagin, leucin). Certain bacteria require albumins of a particular kind as, for example, those contained within blood serum; among these may be mentioned B. diphtheriæ which grows only imperfectly on other culture media. The gonococcus is more obstinate still, requiring human blood serum; and thus far we have failed, through our ignorance of the particular food required, to cultivate the bacillus of leprosy and the spirillum of relapsing fever.

The relation of bacteria to oxygen is very important. While some forms are just as dependent upon this element as are the higher plants and animals, others survive and multiply only in its absence. It is to Pasteur that we owe the discovery of this latter group and the consequent division of bacteria into aerobic and anaerobic forms. The growth of anaerobic bacteria is often associated with active fermentative changes, although such fermentation is no necessary function of their development. Liborius distinguishes three classes of bacteria in relation to their need of oxygen:

1. *Obligatory anaerobes.* The bacteria belonging to this class grow only when oxygen is completely excluded. Among these are found such pathogenic varieties as B. œdematis maligni, B. tetani, B. aerogenes capsulatus, and the bacillus of symptomatic anthrax (Rauschbrand).

2. *Obligatory aerobes,* which grow only if oxygen is freely supplied.

A considerable number of chromogenic and other bacteria belong to this class; pathogenic varieties are, however, wanting.

3. *Facultative anaerobes.* While the bacteria of this class thrive best in the presence of free air, they are nevertheless capable of growing, although more slowly, in its complete absence. This capacity is, however, very variable. The greater number of the pathogenic forms are found in this class.

In the *preparation of culture media* not only must the nature of the various ingredients be carefully studied, but attention must be directed to the proper concentration and reaction of the finished products. Notwithstanding the relatively wide field of selection afforded by the manifold functions of the bacteria, unless some very especial purpose has to be served, the nitrogen and carbon are generally provided in some form of peptone and sugar, while the necessary salts are supplied in beef infusion and by additions of sodium chloride.

The concentration of media is subject to great variation, although in the case of solids the limit is better defined than in fluids. Kappes found that media in which the dried solids amounted to twenty per cent. (of which 7.5 per cent. was agar) were no longer adapted to growth. On the other hand Bolton has described bacteria which not only live but are also capable of multiplying in distilled water. As a rule, however, it may be said that the luxuriance of growth of many bacteria and especially of pathogenic forms is determined by both the concentration and the composition of the media. The addition of glucose facilitates the growth of many bacteria (B. pneumoniæ Friedländer, B. diphtheriæ), while a bacillus closely related to the former (B. capsulatus Pfeiffer) thrives better in the absence of the sugar (Cramer). Glycerin likewise facilitates the growth of B. tuberculosis.

The reaction of the media is of much importance. For the most part a neutral or faintly alkaline reaction is most favorable. Excess of acid is more inhibitory than excess of alkali. A few bacteria demand an acid medium, the acetic-acid bacillus, for example, requiring twenty-per cent. of acid to enable it to grow. Minimal additions of acid are not so injurious as they have usually been considered, and, in general, vegetable acids are borne better than an excess of mineral acids. The typhoid bacillus continues to grow even in the presence of citric and acetic acids, while the anthrax bacillus is able to withstand two per cent. of lactic acid. Advantage may be taken of the influence of this reaction upon growth in the separation of mixed cultures, as, for example, in the case of the cholera spirillum which is able to withstand high grades of alkalinity.

The influence of temperature is of the utmost importance in determining growth. Bacteria agree with the plants in general and with the cold-blooded animals in adapting themselves to the temperature of their environment (poikilothermism). In respect to growth they exhibit the three cardinal points, minimum, optimum, and maximum, common to all living organisms. The conditions, however, vary for different species, no general rule holding for all forms. The various functions, such as growth, motility, sporulation, fermentation, and toxicity, probably have their special optima. A good average is supplied by the growth. Examples are furnished in the following table supplied by A. Fischer:

	Minimum.	Optimum.	Maximum.
Seeds of grains	5–7° C.	29° C.	42.5° C.
Seeds of pumpkin	13.7	33.7	46.2
Bacillus anthracis	14	37	45
Bacillus tuberculosis	30	38	42
Bacillus thermophilus	42	63–7	72
Bacillus subtilis	6	30	50
Bacillus fluorescens liquefaciens	5–6	20–25	38
Bacillus phosphorescens	0	20	38

In general it may be said that saprophytic forms (B. subtilis, B. fluorescens liquefaciens) behave much as do the seeds of the gramineæ; while the pathogenic forms require temperatures approaching that of the warm-blooded animals to promote an abundant growth. Exceptional positions are occupied by the bacillus phosphorescens, an inhabitant of the North Sea, which still multiplies at 0° C., and the thermophilic bacteria, whose optimum of growth approaches the coagulation point of albumin. Along with the variations in growth go other functions. At the minimal temperatures, at which growth is slight, the metabolism is also much depressed, while at the maximal temperatures inhibitory effects are noticed which are only slowly recovered from after a return to optimal temperatures. The weakening of pathogenic bacteria intended for purposes of artificial immunization can be brought about by cultivation at maximal temperatures.

The only bacteria which are influenced by light in a manner similar to the higher plants are the red sulphur bacteria which possess the property of absorbing CO_2. The remaining chromogenic bacteria are subject to the influence of light in a manner similar to that observed in the case of the colorless bacteria. These grow equally well in darkness or in weak diffused daylight; stronger light rays act injuriously, the first effect being to diminish the growth. Still stronger rays, or a more prolonged exposure, may bring about complete cessation of growth and even cause the death of the organisms.

The direct rays of the sun are destructive to bacterial cells and spores in from one to three hours—an effect produced independently of the heat rays.

Preparation of the Culture Media.

Nutrient bouillon. (*a*) *Method of Koch:* Five hundred grams of finely chopped lean beef, free from fat and tendon, is infused in one litre of water at the temperature of the ice chest for twenty-four hours. The fluid is then strained through a coarse towel and the residue pressed until a litre of fluid is obtained. To this are added one per cent. (10 gm.) of dried peptone and 0.5 per cent. (5 gm.) of chloride of sodium (common salt). The mixture is next neutralized or rendered very slightly alkaline by the addition of a suitable quantity of a saturated solution of sodium carbonate, and is then placed in a steam sterilizer over a water bath or directly over the free flame and kept at the boiling-point until the albumin is coagulated and the fluid portion is clear and assumes a pale straw or amber color, care being exercised, if the free flame be employed, to prevent charring of the coagulum. The mixture is then thrown on a filter, and the fluid portion, when collected, will give the nutrient bouillon as ordinarily understood. The subsequent sterilization of this product is to be carried out in an autoclave or by the fractional method (see p. 593).

(*b*) *Commercial meat extracts* may be employed in place of the beef infusion. Although the majority of those on the market will answer the purpose, the preparation known by Liebig's name is, perhaps, the most generally used. It is to be employed in the strength of 2 gm. to the litre of water, the quantity of peptone and sodium chloride added being the same as that given in the previous formula. The subsequent procedures are the same as those already given except that the step of coagulation is inapplicable. The advantage of this method consists merely in the greater convenience of having a ready-made extract as compared with the preparation of the meat infusion and the saving of time which it permits. Bouillon prepared by this method is, however, less valuable as a culture medium and is, therefore, not to be recommended for general use.

(*c*) *Sugar-free bouillon* (Theobald Smith[22]). Fresh beef muscle contains an appreciable quantity of sugar, nor are the commercial meat extracts entirely free from this constituent. It has been found that the presence of sugar in culture media interferes with the exhibition of certain properties of bacteria, while at the same time complicating the fermentation tests commonly carried out with these organisms. To obviate these difficulties several methods have been

introduced for the purpose of obtaining a nutrient bouillon free from dextrose. Beef infusion prepared either by extracting in the cold (see p. 577) or at 60° C. is inoculated in the evening with a rich fluid culture of some acid-producing bacterium (B. coli communis) and placed in the thermostat. Early next morning the infusion, which will be covered with a thin layer of froth, is boiled and filtered; peptone and salt are added and the neutralization and sterilization carried on as usual. It is found that bouillon prepared in this manner is free from sugar and does not, as might have been expected, contain indol.

(*d*) *Spronck's method.* Spronck permits beef to remain in the ice chamber for several days before converting it into nutrient bouillon, in order that in the process of incipient putrefaction the muscle sugar may be broken up. The meat infusion is then made in the ordinary way. Experience has shown this method not to be strictly reliable, as the resulting bouillon may still contain sugar in relatively considerable quantities.

(*e*) *Dunham's peptone solution.* A substitute for bouillon, introduced by Dunham, consists of a solution of one per cent. of peptone and five per cent. sodium chloride in water. The resulting culture medium has been widely employed in testing the indol-producing properties of bacteria. The objection to it is that it constitutes a very poor culture fluid for many bacteria, some failing to grow in it. Its value has now become greatly restricted since the introduction of the bouillon free from dextrose and indol in which bacteria grow well or even vigorously, permitting the indol test to be made even under conditions favorable to the growth of the majority of bacteria.

(*f*) *Peckham's bouillon.* For the purpose of bringing out the indol-producing capacity of certain bacteria, Peckham[27] prepares an alkali-peptone bouillon by the following formula: Beef muscle, 225 gm.; trypsin, 4 gm.; salt, 5 gm.; water, 1 litre. The finely chopped beef muscle, which must be as old as can ordinarily be obtained in shops in order that it may be free from muscle sugar, is put into 500 c.c. of water and the mixture made slightly alkaline with sodium carbonate. The flask containing the mixture is then placed in a water bath at 40° C. and the trypsin is added. After digesting for an hour the fluid becomes decidedly acid, and sodium carbonate is added until alkalinity is reached. Peptonization should be arrested in from one and a half to two hours; otherwise traces of indol may be detected. At the end of this period the mixture is boiled and strained through gauze and then filtered cold through wetted filter paper in order to remove the fat. The salt is now added and the volume made up to one litre. The acidity of the

resulting clear straw-colored filtrate is brought to the right point by the use of the phenolphthalein indicator. Experience has taught that the medium containing such an amount of free acid as to require from 20 to 30 c.c. per litre of a decinormal caustic soda solution to bring it to a point neutral to this indicator possesses the most suitable reaction for the development of certain organisms in the absence of sugar. After sterilization the bouillon is tested for indol and sugar. If these undesirable products are present, the medium is not suited to the purposes for which it was designed, but ordinarily no great difficulty is experienced in obtaining the muscle free from sugar, and indol will be absent unless the tryptic digestion has been permitted to go beyond the prescribed time.

(*g*) *Peptone rosalic acid solution.* Abbott recommends this solution for the purpose of determining acid and alkali production. It consists of Dunham's peptone solution, to each 100 c.c. of which 2 c.c. of a solution of rosalic acid (coralline) of the strength of 0.5 gm. to 100 c.c. of eighty-per-cent. alcohol is added. The filtered and sterilized medium is to be used in the usual manner. It appears as a a rose-colored solution, which, under the influence of acids becomes decolorized, while the presence of alkalies produces an intensification of the original color. Reducing substances, such as glucose, interfere with the reaction.

Nutrient gelatin. In the preparation of this culture medium the bouillon is first prepared according to one of the methods already given. The choice is of no great matter excepting when we desire to obtain a medium which shall be free from muscle sugar. In preparing the nutrient gelatin the neutralization of the bouillon is dispensed with until after the gelatin has been added. As a rule, commercial gelatins contain appreciable quantities of free acid, and therefore require a considerable amount of alkali to bring about complete neutralization. There are, however, in the market preparations of gelatin which are acid-free. The gelatin of the best quality is added to the non-neutralized bouillon in proportions varying from six, twelve, or even fifteen per cent., depending upon the uses to which the medium is to be put, the season of the year, and the climate in which it is to be used. The average strength employed is ten per cent. of gelatin; but for bringing out certain properties (*e.g.*, the swarming of certain bacteria) a weaker solution, containing about six per cent. of gelatin, is better; while in the summer months, in warm climates, a fifteen-per-cent. solution may be needed to obviate the melting of the solidified medium. The gelatin is dissolved in the fluid by heat supplied by either a water bath or the direct flame; in the latter case precautions must be taken to avoid charring of the

mixture. The neutralized and filtered solution is then sterilized, preferably by the fractional method, since the temperature of the autoclave sometimes interferes with the proper congelation of the solution. This method, while generally applicable, sometimes fails to give a perfectly clear product, and in order to obviate this difficulty, egg albumen may be employed (see below), as is constantly done in the preparation of agar-agar.

Potato gelatin. According to Holz, a medium made of potato and gelatin is useful for differentiating B. typhosus. Potatoes which have been cleaned, peeled, and washed are rubbed through a sieve and collected. The juice is then expressed through a cloth and allowed to stand, at a low temperature, for twenty-four hours. This juice, which has now taken on a brown color, is filtered, heated in the steam sterilizer for thirty minutes, and again filtered. After ten per cent. of gelatin has been added to the filtrate, it is heated again in the steamer for forty-five minutes and filtered. The resulting medium has an acid reaction (10 gm. being neutralized by from 1.6 to 3.2 c.c. of decinormal soda solution). In it B. typhosus grows readily, while many other bacteria are inhibited. The addition of 0.05 per cent. of carbolic acid to the medium influences the growth of the typhoid bacillus only very slightly, while it checks considerably the growth of moulds.

Potassium-iodide-potato-gelatin. A useful modification of the potato gelatin has been supplied by Elsner. Its uses are identical with those of the former, and its chief value is found in the inhibition of the growth of many bacteria (excepting B. coli communis) while permitting B. typhosus to develop readily. The colon bacillus grows even more readily than the typhoid bacillus; it colonies are fully developed in twenty-four hours, while those of the typhoid bacillus at this time are, under low powers of the microscope, scarcely visible. At the end of forty-eight hours they are distinguished as small, pale, shining, dew-like points with a very fine granulation which can be readily differentiated from the larger, coarse, brown colonies of the colon bacillus. According to Elsner, potatoes prepared after the formula of Holz are infused in the proportion of 0.5 kgm. to one litre of water. In this the gelatin is dissolved by means of heat. The acidity should be that given by Holz. According to Elsner, 10 c.c. of potato gelatin required 2.3 to 3.0 c.c. of decinormal soda solution, the indicator being litmus. The solution is filtered, one per cent. of iodide of potassium is added, and the mixture is sterilized. As a rule, this medium serves the purpose for which it has been introduced, but, unfortunately, there occasionally develop in it colonies indistinguishable from those of B. typhosus but which in reality

represent totally distinct organisms. It is therefore necessary to employ other tests before accepting as such the "typical" colonies of B. typhosus (see B. typhosus.).

Nutrient agar-agar. Agar-agar, according to Hueppe, although first recommended as a culture medium by Frau Hesse, was brought into general use by Koch. It is obtained from various classes of algæ belonging to the Rhodophycea. As compared with gelatin, agar possesses a number of advantages as a culture medium; it remains solid at a much higher temperature; it is never liquefied by bacteria; in the form of slants it presents a moist surface well adapted for obtaining growths, and when poured upon plates it quickly solidifies without the use of ice. In the usual concentrations it requires the boiling point (100° C.) in order to become liquefied; but once fluidified it does not recongeal until exposed to a temperature below 40° C. Its disadvantages are chiefly dependent upon the difficulty commonly met with in preparing a suitable medium and because in cooling a certain quantity of water is liberated endangering the surfaces of roll tubes. Of less moment is the fact that while liquid the medium is clear and transparent, but in cooling becomes turbid. One method only need be given for preparing the plain agar, inasmuch as extended experience has shown it to be entirely satisfactory and, when the details are carried out carefully, it never fails to give a uniform product.

(*a*) *Schutz's agar-agar.* The principal difficulty in the preparation of nutrient agar-agar has generally been the slowness with which the solution passes through the filter. Various devices, relating partly to the method of mixing or boiling the ingredients and partly to the character of the filter used, have been employed to overcome this difficulty. Hot-water filters of one form or another have been generally considered necessary. By the method of Schutz[28] the hot-water filter is dispensed with and the making of the medium can be accomplished within one or two hours. The requirements are a suitable free flame which has been found in such gas stoves as are supplied with multiple jets arranged in a circle. The boiling is done over a free flame in an enamelled iron vessel having a capacity of two litres or more. In order to make one litre of the finished product, the following proportions are used: Take 1,500 c.c. of water, and 18 gm. of agar-agar, preferably in small pieces, and boil vigorously for thirty minutes. No cover is placed over the vessel and stirring the liquid is unnecessary. The thick white scum which appears floating on the surface should be removed. Two grams of Liebig's beef extract are added while the mixture is boiling. As soon as the solution of the agar is complete, the vessel is removed from the fire and the whole allowed to cool to 60° C. Ten grams

of dry peptone and 5 gm. of sodium chloride are now added and the entire contents of an egg in water (the quantity of water equalling the amount previously evaporated) are thoroughly mixed with the agar solution. The reaction is now tested and is generally found to be too strongly alkaline. By the careful addition of dilute HCl a faintly alkaline or neutral reaction can be obtained. Preferably this neutralization may be made before adding the egg. The saucepan containing the mixture is now put over the flame again and boiled until a coagulum is formed upon the surface and the liquid is rendered limpid and clear. The mixture is now to be filtered through the best white filter paper previously moistened with water. The filtration proceeds rapidly, so that a second passage may be made if desired. If the filtered solution is not fairly clear and transparent, it may be improved by the addition of the white of another egg, the solution being afterward cooked merely long enough to coagulate the egg albumen. At the end of the process one should have 1,000 c.c. of the medium.

If it is desired to prepare the medium with infusion of beef instead of with beef extract, the procedure is modified as follows: One pound of finely cut lean meat, after being digested in 1,500 c.c. of water at a temperature of 60° C. for thirty minutes and strained through a linen cloth or towel, is boiled for five minutes and is then filtered. To this filtrate, in an agate-ware saucepan, agar is added, and the subsequent procedures are the same as those already given. The reaction of the agar made with beef infusion is of course decidedly acid, and the addition of solution of sodium carbonate is necessary.

(*b*) *Gessard's agar.* This agar is recommended as a special medium for restoring to B. pyocyaneus its pigment-producing capacity or maintaining or increasing this capacity when already present. The medium is composed of agar-agar and glycerin, the former being in such proportions as to render filtration of the medium impossible. Gessard recommends the introduction of 0.25 gm. of agar in a test-tube of suitable size. Upon this are poured 5 c.c. of a neutral two-per-cent. solution of peptone and five drops of glycerin. The tubes are then stoppered and the precaution is taken to maintain them for some time in a water bath at the boiling temperature before subjecting them to sterilization in the autoclave, in order to avoid the abrupt disengagement of the air imprisoned in agar-agar. The sterilization, after the agar-agar has been dissolved, is carried on in the autoclave at a temperature of 120° C. for a period of five minutes. The tubes are then solidified in the horizontal position.

(*c*) *Deycke's agar.* Deycke employs a medium containing alkali

albumin for the purpose of isolating more especially the diphtheria bacillus and the cholera spirillum. The formula recommended in the case of B. diphtheriæ consists of one per cent. each of alkali albumin and peptone, sodium chloride one-half per cent., agar-agar two per cent., and glycerin five per cent. The mixture, which always gives an alkaline reaction when first prepared, is carefully neutralized by the addition of HCl, litmus paper being used as an indicator. After neutralization has been accomplished, the medium is again rendered alkaline by the addition of one per cent. of a soda solution of which every three parts contain one part of soda and two parts of water. The mixture, after being permitted to remain for several hours at the room temperature, is then cooked in a sterilizer for a period varying from three-quarters of an hour to one hour. The hot mixture is then filtered through a thin layer of sterilized cotton and transferred to culture tubes. After these have been sterilized, the medium is allowed to congeal with the tubes in a slanting position. Should a perfectly clear medium be desired, so as to be suitable for the preparation of plate cultures, it is advisable to filter through filter paper, for which purpose the hot-water filter is recommended.

For the isolation of the cholera vibrio the mixture recommended consists of alkali albumin 2.5 per cent., peptone and common salt one per cent. of each, gelatin ten per cent. The mixture is neutralized and to it is added two per cent. of the same soda solution as is employed in the agar mixture. The hot solution is filtered and transferred to test-tubes in which the sterilization is carried out by the discontinuous method. Deycke believes that the high alkalinity of this mixture is useful in inhibiting the growth of organisms, other than the cholera spirillum, in the presence of the alkali albumin. In order to render the medium suitable for cultivation at high temperatures a mixture of agar-agar and gelatin is recommended. This consists of agar-agar 2 per cent., gelatin 5 per cent., alkali albumin 2.5 per cent., peptone and NaCl 1 per cent. of each. A suitable quantity of water having been added, the temperature is raised until the gelatin is dissolved. After careful neutralization, the medium is alkalinized by the addition of two per cent. of the soda solution already mentioned. The whole, after being cooked until the agar is dissolved, is filtered through sterilized cotton. The subsequent operations are carried on in the usual manner.

(*d*) *Wurtz's litmus-lactose medium.* Wurtz has recommended a medium consisting of ordinary nutrient agar or gelatin to which has been added two to three per cent. of lactose and sufficient litmus tincture to produce a pale blue color. Bacteria capable of fermenting lactose are distinguishable from those which lack this power. The success

of the medium often depends upon the absence of muscle sugar; sometimes the presence of dextrose in the beef infusion may cause an imperfect reaction. The medium has been much used for the differentiation of the colon and typhoid groups of bacilli. As the typhoid bacillus does not act upon lactose, its colonies or surface growths leave the medium of its original pale-blue color; whereas, the colon bacillus, which produces acid and fermentation in lactose, turns this medium red. The presence of muscle sugar upon which the typhoid bacillus acts with the production of acid, renders the medium unsuited for purposes of differentiation of these organisms.

(*e*) *Guarnieri's agar-gelatin.* The formula for this medium, which is to be recommended more especially for the cultivation of the micrococcus lanceolatus, is as follows:

Meat infusion,	950 c.c.
Sodium chloride,	5 gm.
Peptone,	25–30 "
Gelatin,	40–60 "
Agar-agar,	3–4 "
Water,	50 c.c.

The resulting medium should be exactly neutral. In the preparation of Guarnieri's agar-gelatin it is recommended that the steps in the process given for the preparation of ordinary agar-agar (p. 581) should be followed and then, after the agar has been dissolved, the gelatin is to be added and the whole filtered. This medium is suitable for cultivation at the temperature of the thermostat (37° C.), at which temperature it becomes somewhat softened but does not run. It can be used both for stab and plate cultivations. It is not suitable, as a rule, for slants.

(*f*) *Urine-agar.* Ghon and Schlagenhaufer have recommended a mixture of urine and agar for cultivation of the gonococcus, which has found considerable favor for this purpose. It may be prepared by adding to two parts of sterile two-per-cent. nutrient agar, one part of sterilized urine. It is preferable to collect the urine aseptically; but when this is not possible, freshly voided urine may be sterilized by heat in the usual way.

(*g*) *Glycerin-agar.* Nocard and Roux observed that the tubercle bacillus, which previously had been cultivated exclusively upon blood serum, would grow upon nutrient agar containing from six to eight per cent. of glycerin. The glycerin tends to prevent evaporation of water and thus keeps the surface of the medium moist. It is moreover in itself a nutritious constituent. It is employed as an addition to ordinary nutrient agar in the proportion of five per cent.

(*h*) *Blood serum-agar.* Sterilized blood serum is warmed to 40°

C., inoculated, and then admixed with liquefied agar cooled to 40°–42° C. and poured into plates. The proportions of the mixture may be varied so that the latter may contain any quantity of the serum up to an equal volume with the agar. If slants are desired, the admixture is made and allowed to harden in a suitable position before inoculation.

(*i*) *Blood-agar.* Pfeiffer's medium for the cultivation of the influenza bacillus consists of agar slants over the surface of which sterile human or animal blood is spread in a thin layer.

(*j*) *Capaldi's*[13] *Gelatin-Agar.* This medium is recommended for isolating the typhoid bacillus from dejections, etc.

Distilled water,	1,000 c.c.
Witte's peptone,	20 gm.
Gelatin,	10 gm.
Grape sugar (or mannita),	10 gm.
Sodium chloride,	5 gm.
Potassium chloride,	5 gm.

Filter. Add two per cent. of agar; alkalinize with 10 c.c normal soda solution, boil, filter and sterilize. Colonies eighteen hours old of B. typhosus are small, refractive, and transparent; those of B. coli communis are larger and cloudy (milky).

Blood serum. (*a*) *Koch's method.* The use of blood serum for culture media we owe to R. Koch. He advised collecting the freshly-shed blood under aseptic precautions directly from animals by venesection. The blood is caught in sterile glass vessels which are put aside in a cool place to allow clotting to take place. The serum is drawn off by means of sterile pipettes and transferred at once to sterilized and plugged test-tubes (each containing about 10 c.c.). If the collection has been carried out with all precautions and without accident, subsequent sterilization by heat is unnecessary. Usually it is advisable to use this additional safeguard which, according to Koch, is assured by exposing the charged tubes to a temperature of 56° C., for two consecutive hours on six or eight days. On the last day the tubes, placed in a slanting position, are solidified by raising the temperature to 68° C. A special form of apparatus is employed for these operations. Even when all precautions are taken failures are not uncommon. The temperature of 56° fails to kill resistant vegetative bacteria and spores, so that these, when present, may be only inhibited in their growth for a time, and eventually they may destroy the efficiency of the medium.

(*b*) *Kischner's method.* Blood serum collected with great care is mixed with two per cent. by volumes of chloroform and allowed to remain in sealed flasks for two months. After this time test-tubes, properly sterilized, are charged as in the former case and, if needed,

may be immediately solidified at 68° C., at which temperature the chloroform also is evaporated.

(*c*) *Loeffler's mixture.* This consists of three parts of blood serum and one part of one-per-cent. glucose bouillon. Test-tubes containing this mixture are solidified by heat in the manner given for the preparation of plain serum by Koch's method; or the mixture may be employed in the fluid state. It is recommended especially for cultivating the diphtheria bacillus.

(*d*) *Mallory's method.* By this method a product is obtained which differs from (*a*) in that the serum is not only solidified but is coagulated as well. The translucency of the original medium is lost and the resulting product forms a white opaque layer, that suffices, however, for all of the usual purposes to which this medium is applied. Its advantage lies in the fact that it can be more rapidly prepared. The blood is collected as in Koch's method, although no special precautions need be taken in order to avoid contamination. The blood which is obtained by the Jewish method of slaughter, namely, by severing the carotid artery, is best, inasmuch as it clots more readily. Three parts of the separated blood serum are mixed with one part of glucose bouillon and the mixture is then run into test-tubes. The presence of a moderate number of red blood corpuscles does not interfere essentially with the end product. The tubes containing the requisite amount of the mixture (3–4 c.c.) are best subjected to the action of the heat while in the slanting position in order that the solidified mixture may offer a smooth slanting surface for culture purposes. The coagulation is effected either in the hot-air sterilizer by packing the tubes on their sides or better in the blood-serum coagulator of Koch, the tubes being arranged in several layers upon the shelves of the apparatus. The hot-air sterilizer will be found to give excellent results if the temperature be carefully watched and the process controlled from time to time. The temperature in the hot-air sterilizer should not be allowed to exceed 90° C., nor to fall below 85° C., the door being kept closed. The tubes are exposed in this sterilizer for a period of from two to three hours, which is the usual time required for the coagulation for those forming the middle layers. But since the lower layers are liable to become overheated during this time, it is desirable to employ a false bottom or to have one or two layers of the lowest tubes empty. The blood-serum coagulator gives perhaps more satisfactory results, although with it more time is consumed. The temperature of the interior should be kept at about 95° C. The average time of exposure is three hours.

The essential points to be looked to are the complete coagulation of the mixture and the assurance that the medium in the tubes has

become firm and solid. If these are not obtained, bubbles and cavities will form and destroy the smoothness of the surface of the medium when it is subjected to the final steam sterilization. The coagulated tubes are placed in wire baskets with the cotton stoppers uppermost and subjected to steam sterilization by the fractional method. The use of the autoclave is inadvisable, inasmuch as the great pressure exerted in it produces breaks, rifts, and cavities in the medium.

(*e*) *Nuttall's method.* For certain purposes it is desirable to obtain blood perfectly free from bacterial contamination. In order to accomplish this, recourse may be had to the method introduced by Nuttall, in which the blood is collected in bulbs (Fig. 2), introduced into the exposed femoral or carotid arteries. The distal portion of the artery having been tied off, the end of the sterile bulb is introduced through a small slit made in the proximal portion of the vessel, the vessel wall being tightened around the edge of the tubular portion of the bulb by means of a second ligature. The clamp which has controlled the blood during this operation having been removed, the blood is now forced into the bulb by its own pressure. After a sufficient quantity has been collected, the bulb is removed, and its end having been sealed in the flame, it is laid aside in order to allow coagulation to occur and the serum to separate. The clear serum is then removed, as required, through the upper large opening of the bulb.

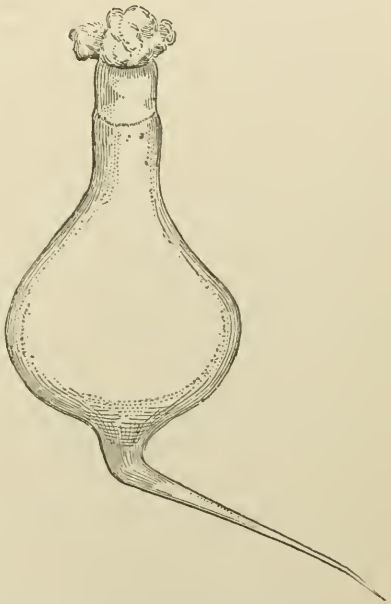

FIG. 2.—Nuttall's Bulb.

If larger quantities of sterile blood serum are desired, they may be obtained from animals, such as the dog and sheep, by exposing the femoral artery and introducing a cannula made of glass and bent at appropriate angles so as to deliver the blood directly into a sterilized glass vessel. A large dog will in this way yield from 1,000 to 1,500 c.c. of sterile blood, the separated serum being collected in the usual way.

Potato. (*a*) *Koch's method.* The original method of Koch consists in first selecting the potatoes, which should be of the kind used

for salads, that is, those which do not burst open in the boiling, and which after being cooked and cut present a smooth, shining, and not a mealy surface. These potatoes are first scrubbed vigorously with a stiff brush to remove all extraneous dirt and other particles. The eyes and other imperfections are then removed with the end of the potato-knife, held at right angles to the surface. If there are present diseased areas extending deeply into the substance, the potato is to be discarded. Care must be exercised to leave the normal epidermis as nearly intact as possible. The potatoes are rinsed and placed in a 1:1,000 sublimate solution (sublimate 1, hydrochloric acid 5, water 1,000) for one-half to one hour. This is done in order to destroy as many as possible of the moist earth bacteria. The potatoes are now steamed for half an hour in a vessel with a perforated bottom. After removal and sufficient cooling they are taken out, by grasping them between the thumb and index finger, the hand having been previously sterilized in sublimate solution, and halved in the longitudinal direction with a potato knife recently sterilized by heat. The halves are permitted to fall apart so that the cut surfaces are exposed in special glass dishes, sterilized with sublimate, which constitute moist chambers. Such a chamber consists of a pair of glass dishes, the upper fitting easily over the under. The lower half should have a diameter of 20 cm. and a depth of 6 cm., and the bottom is covered with a piece of filter paper moistened with 1:1,000 sublimate solution.

(*b*) *Bolton's method* is to be recommended as affording simplicity and avoiding the ordinary dangers of contamination. The potatoes are freed of their coarser dirt by thorough scrubbing with water and brush. By means of a cork-borer, or an instrument especially designed for the purpose, cylindrical columns are punched out of such a size as to be comfortably received by the test-tubes to be employed. Each such column is then divided in an oblique direction so as to afford two slanting surfaces, or if, as is more usual, it is not possible to obtain two portions from the same cylinder, one edge is cut so that the slanting surface runs from about the junction of the first and second thirds of the cylinder diagonally to the opposite side. The potato cylinders prepared in this way are to be left in running water for several hours or over night in order to avoid the discoloration which otherwise ensues upon sterilization. They are now placed in cleansed, sterilized test-tubes with the slanting surface upward; after the corks have been returned, the tubes are to be sterilized by the fractional method, or better, by the use of the autoclave for a period of from twenty to twenty-five minutes.

Potatoes have often a faintly acid reaction which is objectionable in the cultivation of certain bacteria. To overcome this, they may

be immersed in a one-per-cent. soda solution before sterilization—the so-called "soda potatoes." If it is desired to increase the acidity, a one-per-cent. solution of acetic acid may be employed.

Milk. (*a*) *Fresh milk.* Milk makes an excellent medium for the growth of many bacteria and it serves an especially valuable purpose in differentiating certain closely allied varieties. The changes which milk undergoes in the presence of acids and ferments bring out striking reactions. Fresh milk which has been kept for twelve hours at the temperature of the ice chest is deprived of its creamy layer and then transferred to recently sterilized test-tubes, about 8 to 10 c.c. being put into each tube. Sterilization may be effected by the fractional method, but on account of the presence of highly resistant, spore-bearing organisms, it is preferable to use the autoclave.

(*b*) *Litmus milk.* The addition of an infusion of litmus to milk prepared as above, while not interfering with its cultural properties, is of additional value on account of the indication of acid or alkali production. A sufficient quantity of tincture of litmus is added to give a pale blue color. Sterilization is carried on in the usual way, and during this process the color may temporarily be lost owing to the heat employed. A more complete and durable decolorization of the litmus is brought about by certain reducing bacteria. Under these circumstances, however, the color can be restored by agitation and admixture of atmospheric air, and will be of the original tint or more pronouncedly blue or red depending upon whether an acid or alkali or neither has been produced by the bacteria along with the decolorization.

Eggs. (*a*) Hueppe has employed fresh eggs for cultivating bacteria. The eggs are first carefully cleansed with soap and brush; one end is then washed in sublimate, rinsed in sterile water, and dried with sterile cotton. A small opening is now made with a red-hot needle, through which the inoculation is conducted. The opening is sealed with sterile silk paper and collodion. Another method (Gunther), instead of washing and drying, is to burn the end with a hot knife, then to make an opening with the hot needle and to proceed as before. Hueppe believed that the growths so obtained were anaerobic; but this is now known to be an error, as air enters through the shell.

(*b*) Zorkendorfer employs the egg contents freed from the shell. He opens the fresh eggs and pours the yelk back and forth in the halved shells until all the albumen is separated. This is then poured into an Erlenmeyer flask which is then set in ice water. The yelk is now placed in the mouth of the flask and the atmospheric air presses it through the neck without rupture. The flask is now closed with

sterilized cotton and sterilization is effected by the fractional method employed by Koch for blood serum.

(*c*) *Egg-yelk agar* or *bouillon* is recommended by Capaldi for cultivating refractory bacteria (B. tuberculosis; B. diphtheriæ). Fresh egg yelk is added to completed agar or bouillon. Sterilization at high temperatures is to be avoided.

Sugar media. The addition of several sugars (glucose, lactose, saccharose) to agar, gelatin, and bouillon is found useful in the cultivation and differentiation of bacterial species. The sugar must be of chemical purity and the quantity employed varies from one to five per cent., the average being two per cent. In order that accurate results may be obtained care should be exercised to employ as a base only media from which all muscle sugar has been eliminated (see sugar-free bouillon, p. 577).

Albumin-free culture media. (*a*) Pasteur had shown, in the year 1858, that yeasts would grow in fluids containing no albumin, and that they, in common with the higher plants, were capable of acquiring the nitrogen necessary for their growth from ammonia. The formula for Pasteur's fluid is as follows:

Distilled water,	100 parts.
Pure saccharin,	10 "
Tartrate of ammonium,	1 part.
Ash of 1 part yeast cells,	about 0.075 part.

According to Pasteur, the carbohydrates are supplied by the sugar, the nitrogen by the ammonium tartrate; whereas, the mineral substances, also necessary, are contained in the ash of the yeast cells.

(*b*) Ferd. Cohn modified this solution so as to render it suitable for the growth of bacteria. The formula given by him is:

Distilled water,	20.0 c.c.
Phosphate of calcium,	0.1 gm.
Sulphate of magnesium,	0.1 "
Tribasic phosphate of calcium,	0.01 "
Tartrate of ammonium,	0.2 "

(*c*) The medium, however, which is most useful for the cultivation of a large number of pathogenic bacteria is that introduced by Uschinsky. It consists of the following ingredients:

Water,	1,000 parts.
Glycerin,	30–40 "
Sodium chloride,	5–7 "
Calcium chloride,	0.1 part.
Magnesium sulphate,	0.2–0.4 "
Potassium diphosphate,	2–2.5 parts.
Ammonium lactate,	6–7 "
Sodium asparaginate,	3.4 "

(*d*) This mixture has been simplified by C. Fraenkel.[14] He employs a solution composed of:

Sodium chloride,	5 parts.
Potassium diphosphate,	2 "
Ammonium lactate,	6 "
Asparagin,	4 "
Water,	1,000 "

The mixture is rendered perceptibly alkaline by means of soda solution. The several artificial albumin-free culture media are sterilized in the same manner as gelatin, agar, etc., and are then used as fluid media.

For the purpose of cultivating moulds the use of soft mush made of bread is recommended. This is prepared by introducing dried and comminuted bread into test-tubes from 3 to 4 cm. in height. Water is then added until the bread is completely wetted through and the tubes are then stoppered and sterilized in the usual manner. Inasmuch as the bread mixture has an acid reaction it does not serve as a culture medium for bacteria.

Titration of Culture Media.

One of the important steps in the preparation of culture media is their proper neutralization. Several methods are in vogue. The nutrient medium—bouillon, gelatin, or agar-agar—may be brought to the neutral point, as indicated by litmus paper, through the addition of acids or alkalies according to the reaction of the original fluid. As a rule, culture media in their raw state are acid and require the addition of an alkali to bring about neutralization.

Until within the last few years litmus has been employed almost exclusively as an indicator; but experience has now shown that it is not reliable, inasmuch as errors are introduced through the presence of acids and acid salts to which the litmus does not react. In the presence of an excess of acid, should litmus be used as an indicator, it is better to employ sodium carbonate for the purpose of neutralization rather than the solution of the hydrate, since the small amount of carbonic acid liberated from the former compound necessitates the use of a slight excess of alkali which, to a certain extent, obviates the lack of susceptibility of the indicator to vegetable acids and acid salts. A more uniform and correct method of neutralization of the media, however, can be obtained by substituting phenolphthalein for litmus as an indicator. The main advantage of the use of this compound is derived from the property possessed by it of taking into account the reaction of weak organic acids and of organic compounds which have an amphoteric reaction, but in which the acid

character predominates. A similar susceptibility is to be found in turmeric, but since this color changes less readily, it is inferior to phenolphthalein.

The question of the proper reaction of media for the cultivation of bacteria, and of the methods for obtaining this reaction, has been discussed by Fuller.[15] Burettes graduated into tenths of a cubic centimetre are employed. The solutions required are:

1. 0.5 per cent. solution of commercial phenolphthalein in fifty per cent. alcohol.
2. $\frac{N}{20}$ solution of sodium hydroxide.
3. $\frac{N}{20}$ solution of hydric chloride.

Solutions Nos. 2 and 3 must be carefully made up and must correspond with the normal solutions to be referred to. Inasmuch as solutions of sodium hydroxide are subject to some degree of deterioration from the absorption of carbon dioxide, and the consequent formation of sodium carbonate, it is well to place in the bottle containing the sodium solution a small amount of calcium hydroxide, while the air entering the burettes or the supply bottles should be made to pass through a U-tube containing caustic soda to extract from it the carbon dioxide.

All the ingredients having been dissolved, the medium to be tested is brought to the prescribed volume by the addition of distilled water to replace that lost in boiling, and is thoroughly stirred. Five cubic centimetres is then transferred to a six-inch porcelain evaporating-dish. Next 45 c.c. of distilled water is added to the 5 c.c. of fluid and the whole is boiled for three minutes over a flame.

One cubic centimetre of the solution of phenolphthalein (1) is then added, and by titration with the required reagent (Nos. 2 and 3) the reaction is determined. In the majority of instances the reaction will be found to be acid, so that sodium hydroxide is the reagent most frequently required. For purposes of greater accuracy this determination should be made not less than three times, the average of the results obtained being taken as the degree of reaction.

One of the most difficult things to determine in this process is exactly when the neutralization point is reached as shown by the color developed, and to be able in every instance to obtain the same shade of color. In bright daylight the first change that can be seen on the addition of the alkali is a very faint darkening of the fluid, which, as the alkali is increased, becomes a more evident color and develops into what might be described as Italian pink. A still further addition of the alkali suddenly brings out a clear, bright pink color—the reaction which we should always aim at obtaining. The

titrations should be made quickly and in the hot solutions to avoid complications from the presence of carbon dioxide.

The next step in the process is to add to the bulk of the medium the calculated amount of reagent, either acid or alkali, as may have been determined. Normal solutions of sodium hydroxide or of hydric chloride are used, and after being thoroughly stirred, the fluid thus neutralized is again tested in the same manner as before to ensure the proper reaction. When neutralization is to be effected by the addition of an alkali, it sometimes happens—for some reason as yet not understood—that after the calculated amount of normal solution has been added, the second test by titration will show the medium to be still acid to phenolphthalein even to the extent of from 0.5 to 1 per cent. When this happens, the reaction of the medium must be brought to the desired point by the further addition of sodium hydroxide, and the titrations and additions of alkali must be repeated until the medium has the desired reaction. If, after the prescribed period of heating, the medium is again slightly acid (usually about 0.5 per cent.), the fluid is to be filtered and the calculated amount of acid or alkali added to change the reaction to the one desired.

In view of the complication of this method of neutralization, for ordinary purposes and to obtain the growth of the usual bacterial species litmus is still very commonly used as an indicator, although it is highly desirable that greater uniformity of culture media than can be obtained in this way should be introduced.

Methods of Sterilization.

1. *Fractional method.* This is carried out (*a*) in an atmosphere in which steam replaces the air; and (*b*) in an incubator or water-bath at some temperature not less than 60° C. With the first method, sterilization is employed on three successive days. By the second method a longer period is required, it being advisable to repeat the exposure to heat on six or seven successive days. The principles underlying the method of fractional or discontinuous sterilization are briefly as follows: At temperatures varying from 60° to 100° C. many vegetative and some spore forms of bacteria are destroyed, whereas at these temperatures more resistant spores remain unaffected, or at least remain still capable of development. In the intervals between the successive sterilizations the resistant spores are believed to germinate, and these vegetative outgrowths are then destroyed in the next successive sterilization. By the end of the third sterilization at a temperature of 100° C., and at the end of the sixth or seventh when a lower temperature is employed, all living organisms will have, as a

rule, been killed. The time of exposure to these several temperatures varies with the degree of heat employed and with the substance to be sterilized. In streaming steam an exposure for from fifteen to thirty minutes is sufficient, whereas at the lower temperature longer exposures, even up to one hour and more, are advisable.

2. *Continuous method.* Organisms which resist a temperature of 100° C. may be destroyed in a short time at 115° to 120° C. in a single exposure. For this purpose some form of apparatus in which superheated steam can be brought into contact with the object to be sterilized is necessary. Such a contrivance is supplied in the autoclave. At a pressure of two atmospheres a temperature of 120° C. is obtained, which usually suffices for the sterilization of apparatus and media after an exposure of from five to fifteen minutes. The autoclave is applicable to most culture media. Those containing gelatin and composed of blood serum cannot, however, be sterilized with safety in this apparatus. The advantages which this method offers are (1) economy of time, and (2) preservation of the media from contamination by the presence of resisting spore-bearing bacteria that have not succumbed even to the successive sterilizations mentioned before. Experience has shown that B. subtilis at certain seasons of the year abounds in the water and in the air, and that, having gained access to the culture media, the ordinary fractional methods of sterilization do not suffice to destroy its spores, so that serious errors have sometimes been made on account of the development of these organisms in media supposed to have been sterilized.

The Cultivation and Study of Bacteria.

Forms of Cultures.—Distinctions are drawn between stab (stick), stroke or slant, fluid, and plate cultures. The differences are explained in part by the respective names. In making a stab culture a solid medium, *e.g.*, gelatin or agar-agar, is inoculated by introducing the living bacteria by means of a straight needle plunged into it and carried to the bottom of the tube. In a stroke culture the inoculated needle is drawn over the slanting surface of a tube containing a solid culture medium. In order to obtain this slanting surface the tube is fixed in an oblique position during the congelation of the medium. Fluid media such as bouillon, Dunham's solution, milk, etc., are inoculated by transferring into them living bacteria from some given source by the use of a straight needle or the loop (Oese). Inoculation of potatoes and other solid culture media are made by the stroke method already described. The several methods of cultivation mentioned are applicable to the study of bacteria, in the so-called pure

cultures; and many of the properties of the various organisms are brought out in the course of their development upon these several media.

For the purpose of studying the individualized colonies, developing presumably from a single microorganism, and also for separating mixtures of several bacterial species, plate cultures are employed. These are now usually made in Petri's dishes, which have entirely replaced the glass plates introduced by Koch. Plate cultivations are made in solid media, the melted medium being inoculated with living bacteria properly diluted, after which the liquefied mass is poured into the plate and allowed to congeal. To exclude atmospheric germs the cover is immediately replaced after the fluid has been introduced. After the congelation of the fluid the plate is placed in the thermostat. The practical routine of making plates is as follows: Either gelatin or agar-agar is chosen, and three tubes are melted in the water bath. The gelatin melts readily at 25° C., and remains liquid even at a much lower point. In order to melt the agar the boiling-temperature is required, but once melted, this medium remains fluid at temperatures above 36° to 40° C. The gelatin tubes can be inoculated immediately after melting; the agar tubes must be cooled to a temperature approaching 40° C. Tube No. 1 of either series is inoculated with bacteria from any given source. For example:

(*a*) From a previous pure culture.

(*b*) From mixtures of bacteria, as in water, earth, etc.

(*c*) From unknown substances, as in making cultures from animals dead of supposed bacterial diseases, and from exudates, etc.

If a previous culture is employed, tube No. 1 should be inoculated with a straight needle so that not too many bacteria are carried into it. If surface water containing small numbers of bacteria is to be examined, larger quantities up to a cubic centimetre may be employed. If organs of animals are to be studied, then several loops of the juice from such organs are introduced into the first tube. From the first tube the remaining two tubes of the series are inoculated. Three loops are introduced from tube No. 1 into tube No. 2, and then, the needle having been burned in the flame, three loops are introduced from No. 2 into No. 3. The several tubes are now poured into their respective Petri dishes, and after congelation are incubated. The results vary according to the number and viability of the bacteria introduced, but, as a rule, it is found that plate No. 1 contains large numbers of colonies, too closely packed to afford opportunities for the full development of individual colonies; plate No. 2 contains separated colonies; whereas plate No. 3 probably contains only a small number of colonies well separated and adapted for isolation.

Occasionally plate No. 3 fails to show any development. In this case, of course, too few living organisms have been introduced into the first tube, but as a matter of experience this excessive dilution seldom occurs.

One of the objects of this method is to secure separated colonies, and this aim may be attained by a somewhat simpler procedure. Several tubes of slanted gelatin or agar-agar are inoculated in succession with the bacteria carried in upon a straight needle or loop. The surfaces are rubbed with a platinum needle which is not recharged during the seeding of the several tubes employed. The first tube of this series usually gives a diffuse growth from a large amount of inoculated material left upon it, whereas the surfaces of the third or fourth tube will exhibit only minimal growths and even separated colonies. Another modification consists in the inoculation of the condensed water of slanted agar tubes, which collects in the angle between the medium and the glass, and then permitting this to flow over the surface of the medium. This method is said to suffice also for the separation of mixtures of bacteria so that several kinds of colonies may appear as in the Petri plates. These are distinguished by form, size, color, etc. The plate method also serves for the purpose of indicating in such mixtures the numerical relations of any form, the end plate containing the predominating organism, alone or at any rate in the greatest numbers.

Aerobiosis and Anaerobiosis.

Some bacteria are capable of growth only in the presence of free oxygen, others demand that oxygen shall be excluded, while still others are capable of developing both in the presence and in the absence of oxygen. Bacteria which develop only in the presence of oxygen are spoken of as obligatory aerobic, those which grow only in the absence of oxygen are termed obligatory anaerobic forms, while those which are indifferent in respect to oxygen are distinguished by the designations of facultative aerobic or anaerobic organisms. Aerobic as well as anaerobic bacteria in the course of their growth utilize oxygen. The aerobic varieties absorb it from the air, the anaerobic obtain it by decomposition of the culture medium. All bacteria, so far as they have been studied, liberate in their growth carbon dioxide. The ordinary methods of cultivation of bacteria apply to aerobic and facultative aerobic forms, while in the case of the anaerobic bacteria special methods are necessary. Of these the following may be mentioned:

(*a*) *Replacement of oxygen.* For this purpose hydrogen is most commonly utilized, and is passed into the fluid or fluidified culture

medium until the oxygen has been entirely displaced. The tubes are then sealed to prevent further entrance of atmospheric air. Special tubes have been devised for anaerobic cultures. That known as the Liborius tube is, perhaps, the one most generally employed. The manner in which the hydrogen is passed into the medium is indicated in the figure (Fig. 3). After the atmospheric air has been wholly driven out, leaving behind an atmosphere of hydrogen, the ends of the tube are sealed up. The same result can be accomplished if an ordinary test tube is drawn out at a point about two-thirds of its height so as to produce a constriction that will permit of the passage of a pipette of small calibre. Such a pipette connected with a hydrogen apparatus is introduced into the medium already inoculated and the hydrogen is allowed to bubble through the fluid. When the air is entirely displaced the pipette is withdrawn and the drawn-out portion of the tube is sealed in the flame. There is some danger of fracture of the sealed portion of the tube if it be too suddenly cooled, and to avoid such an accident the end is passed through the ordinary flame of the Bunsen lamp sufficiently to render the cooling-process gradual—a result that is assisted by the deposition of the carbon upon the glass. Novy's method and apparatus may also be employed (p. 599). Many anaerobic bacteria can be cultivated in the following manner: Test tubes three-fourths filled with agar-agar or gelatin are exposed to the temperature of boiling water in a bath until the medium is completely melted and practically all the air has been driven out. After being rapidly cooled off, by means of ice, to a temperature between 36° and 40° C., these tubes are inoculated and then plunged into cracked ice to bring about rapid solidification. A second set of uninoculated tubes containing melted medium being in readiness, after the solidification of the first this melted material is poured on the surface and then a second cooling is resorted to. In this way air, and therefore oxygen, is excluded from the lower portions of the tubes for a considerable period.

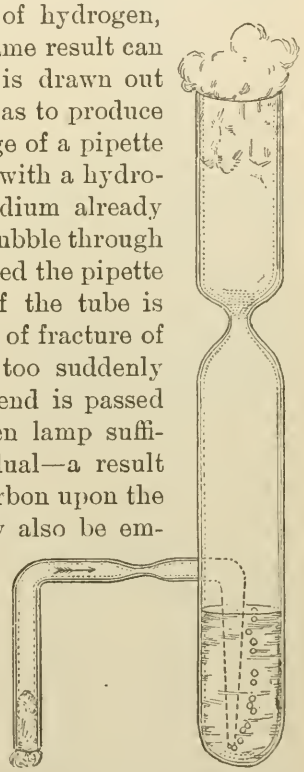

Fig. 3.—The Liborius Tube, showing the way in which the hydrogen is passed into the medium in anaerobic cultures.

(*b*) *The use of reducing agents.* Certain substances when introduced into culture media have the property of fixing the liberated oxygen. Glucose in the proportion of two per cent. will sometimes

effect this, while formate of sodium in percentages varying from 0.3 per cent. to 0.5 per cent. accomplishes the same result even better. From the media containing these chemicals atmospheric air should be excluded by boiling, and the inoculated tubes should be quickly cooled by means of ice. The fermentation tube can also be used for the cultivation in fluids of anaerobic bacteria, the sugar facilitating their development in the closed ends of the tube. Theobald Smith also recommends placing a fragment of some sterile organ (from rabbit, etc.), into the closed portion of the tube before inoculation: the air need not be excluded by boiling.

(*c*) *The absorption of atmospheric oxygen* (*method of Buchner*). Alkaline solutions of pyrogallic acid possess the property of absorbing oxygen. If into a vessel capable of being tightly closed and of receiving several test tubes, there be introduced a gram of pyrogallic acid, 1 c.c. of liquor potassæ, and 10 c.c. of water, and the cover be immediately replaced upon the vessel, in a short time the free oxygen of the atmosphere will be largely absorbed and no longer remain free in the tubes. The solution meanwhile becomes dark in color. The whole apparatus may be placed in the thermostat. Only the most obligatory anaerobic microorganisms fail to grow under these conditions.

(*d*) *Nikiforoff's method.* This modification is adapted to the study of bacteria in hanging drops. The cupped slides are first surrounded as usual with vaseline, upon which the cover glass containing the infected material is placed. In applying the cover glass care must be taken that along one edge the ring of vaseline is imperfect, and at this point a strong watery solution of pyrogallic acid is introduced by means of a platinum loop. By capillary attraction this drop is disposed in the form of a semicircle at the point of union of the cover glass and the excavation of the slide. From the opposite side of the cover glass, a drop of liquor potassæ is introduced in the same manner, after which the cover slip is displaced until the cup in the slide is completely closed. During the rearrangement of the cover slip the two solutions, at first separated, are brought together, but without extending as far as the bouillon drop containing the bacteria. The mixture now brings about the immediate absorption of the oxygen, after which the growth of organisms can be studied directly under the microscope.

Study of Anaerobic Plate Cultures.—Several devices have been introduced for the purposes of studying anaerobic plate cultures. Two only need be mentioned here. Vessels of suitable sizes are employed, containing chambers for the purpose of holding the alkaline pyrogallic mixture of Buchner. Into these Petri's plates are introduced.

The absorption of oxygen which follows is usually sufficient to permit the development of many forms of bacteria. A better method is that of Novy, in which hydrogen is passed for an hour through an apparatus capable of being sealed air tight. Figure 4 will explain the manner of employing this apparatus. It is also adapted for incubation in the thermostat.

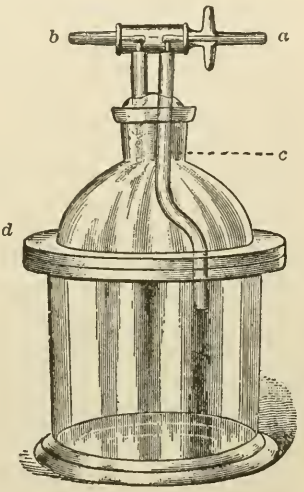

FIG. 4.—Novy's Apparatus. The cock *a b* is covered with a thin layer of vaseline and then introduced into the cylinder. Hydrogen, from a generator, is now passed into *a*, the cock being so turned as to permit the opening to communicate with the tube *c*; air escapes at *b*; after the hydrogen has been passed into the jar for a period sufficiently great to exclude the air, the cock is turned half around, to close the openings, and the hydrogen apparatus is detached; the rim *d* is also to be vaselinized and preferably clamped; the entire apparatus is placed in the thermostat.

THE MICROSCOPICAL EXAMINATION OF BACTERIA.

(*a*) *Unstained preparations* of bacteria occurring in crude materials of all sorts, as well as in pure cultures, can be examined under the natural conditions in which they grow by placing suitable quantities of the containing substance upon the cover slips and using high powers of the microscope. In making such examinations the exact procedure will depend upon the object to be attained. If it be intended merely to demonstrate the presence or absence of bacteria, a thin layer of the crude material is prepared, covered with a film, and examined with the oil immersion lens. Nothing further than the proper management of the light entering through the condenser is required. Besides general form and grouping, motility can also be demonstrated by this means. A special method for clearing up the question of motility is by the use of the hanging drop, for which purpose cupped slides are employed. A ring of vaseline surrounds the cup upon which the film is placed, with the inoculated side down, so that the drop of fluid containing the suspended bacteria projects into the hollow depression of the slide.

(*b*) *Stained Preparations.*—Although quite a number of important facts can be gathered from the study of unstained specimens of bacteria, great advances have been made in our knowledge, especially with regard to their morphology, since the introduction by Weigert and Ehrlich of the anilin stains. At present, although the number of different stains is legion, only a few thus far have been found to possess properties such as renders them especially adapted for some

one definite purpose (see page 603). The staining of bacteria upon film preparations is best carried out by following the method originally introduced by Koch. Minute quantities of a suspension, derived from crude materials containing bacteria, or from pure cultures, are spread in a thin layer upon cover slips. These films are permitted to become air dry, and are then grasped by a special forceps, and passed three times through the Bunsen flame. This passage must not be made too rapidly or the bacteria will not be fixed; on the other hand, if it be made too slowly, the films will be burned. Thus prepared they are now readily stainable by various anilin colors, some difference being observed according to individual peculiarities. In making film preparations from pure cultures, some care is needed in order to avoid obtaining too many or too few bacteria upon the surfaces. It is also desirable to use a minimum of force in spreading the material on the glass in order to preserve as far as possible the grouping of the organisms. If such cover slip or film preparations are made from fluid cultures, *e.g.*, bouillon or Dunham's solution, there is less danger of transplanting too great a number of bacteria. On the other hand, fluid cultures rarely give as sharp, clean pictures as suspensions from solid media, and, besides, are very liable to give rise to precipitation upon the glass. The best method of procedure is as follows: The cover glass is first carefully cleansed especially from grease, and upon it a small drop of sterilized water is placed by means of the loop. The inoculation is then made from a solid culture with a straight needle, and the fluid is carefully spread over the surface, where it is allowed to dry before heating and staining. Somewhat better defined effects are obtained by treating the cover-slip preparation for a moment with a dilute solution of acetic acid (one part of glacial acetic acid to one thousand parts of water) before mounting. In this way the albuminous elements introduced from the culture medium are decolorized, while the bacteria are unaffected. The use of this decolorizing bath cannot be too highly recommended in the study of film preparations made directly from the excreta and secreta derived from the animal body. The acid mixture, while tending to remove the color from the albuminous material existing between the body cells, also serves to differentiate nucleus and protoplasm, so that it not only gives a clear picture of bacteria contained among the cells in the film, but also brings into clear view those bacteria which may chance to exist within the protoplasm of cells, and even within their nuclei.

(*c*) *Impression Preparations.*—The use of impressions is limited to surface colonies upon plate cultures. A cover slip is carefully cleaned and sterilized in the flame; after being allowed to cool it is

placed on the surface of the medium over the colony, and pressed gently down so as to come into contact with it. In making this manipulation care should be taken that in covering the colony all air is excluded. This may be accomplished by placing one edge of the cover slip at a suitable distance from the colony and then permitting the film to descend upon it in such a way that the angle formed by the surface of the medium and the slip becomes gradually more acute until it is entirely obliterated and the cover slip lies horizonally in close contact with the colony. Gentle pressure having been exerted, the edge opposite to that which was first depressed is gradually raised by means of a sterile needle or forceps until the cover slip is finally removed. It will now be observed that the whole or a considerable part of the colony has adhered to the cover slip. This is allowed to become air dry, and then heated in the flame for the purpose of fixation, after which staining by any suitable method can be carried out. This procedure gives us much information concerning the grouping of bacteria in the normal colony growth. It is particularly applicable to B. anthracis, proteus, etc.

Staining of Bacteria.

(*a*) *In Film Preparations.*—For obtaining stained preparations of bacteria it is advisable to spread the suspended organisms upon cover slips, although slides may also be used for this purpose. The advantage of the use of cover slips consists in the higher magnifications to which they can be exposed—a consideration of some importance, especially when it becomes desirable to photograph the bacteria. On the other hand, for the rapid examination of several cultures or several different colonies, the suspensions may be made directly upon the glass slide. For this purpose four, five, or six droplets of sterile water placed upon a clean slide, are inoculated in succession with an equal number of cultures or colonies in the manner given for the preparation of ordinary cover-slip preparations. A number or letter, to indicate the origin of each film, can be placed beside it upon the glass with a wax pencil. The films upon the slide are now fixed and stained as in the manner directed for ordinary cover-slip preparations. After the films have been dried, immersion oil is placed directly upon them and the examination can be made. If it is desired to make a permanent preparation of any or several of the films, the immersion oil is removed by blotting, or by dissolving it in xylol, after which balsam and cover-slip are applied in the usual manner.

In the staining of bacteria it is found that these organisms present several properties which must be taken into account. In this connection subdivisions may be made as follows:

(1) Bacteria which stain readily in alkaline or basic solutions of anilin dyes.

(2) Bacteria which are stained with difficulty with the foregoing dyes, requiring either long immersion or some special procedure, such as the use of a mordant or heat, but which, when once stained, retain the dye with equal pertinacity.

(3) Bacteria which, while stainable by means of anilin dyes as applied in (1), are also stainable after the application of mordants, such as are used in the methods of Gram and of Weigert.

The greater number of pathogenic bacteria are stainable by method (1), while the bacillus of tuberculosis and of leprosy, the streptothrix of actinomyces, and some other bacteria require the conditions mentioned in (2). These organisms also stain by (3), but the especial value of that method lies in the differentiation between allied morphological forms through their different behavior in respect to decolorizing agents after the action of mordants.

The mordants which are generally employed in order to stain B. tuberculosis and certain others are anilin oil and carbolic acid. The mordant used in the methods of Weigert and Gram is iodine dissolved in water by the aid of iodide of potassium. After the application of the mordant which follows that of the stain, decolorization is effected either with alcohol (Gram's method), or by means of anilin oil (Weigert's method). These procedures are employed to stain not only the bacilli of tuberculosis, leprosy, anthrax, and emphysematous gangrene, but also several streptothrices and the pyogenic cocci. The remaining pathogenic bacteria for the most part refuse entirely to be stained in this way. A notable exception to this rule is found in B. proteus, individuals or races of which are stainable in this way, whereas others are entirely refractory.

(*b*) *The Staining of Bacteria in Tissues.*—The tinctorial properties of microorganisms contained within the tissues are somewhat the same as those in film preparations, although it may be broadly stated that bacteria are less readily colored in tissues than in films. As a preliminary to the staining, thin sections are of course required, and the tissues are for this purpose hardened and embedded either in celloidin or in paraffin. Fairly satisfactory results, however, can be obtained by simply boiling the tissues containing the microorganisms, and then sectioning them on the freezing microtome. The sections are carried first into water, then into graded alcohols, after which they are stained in the same manner as tissues hardened by the usual methods.

Formulæ for Staining Solutions.

(1) *Concentrated Aqueous Solutions.* — Various anilin stains are employed in concentrated aqueous solutions made by saturating distilled water with the desired anilin color. These solutions are employed as such, or are diluted at the moment of using to any desired degree. They should either be prepared fresh or at most should not be kept longer than a few days or a week or two, on account of their tendency to undergo chemical changes which interfere with their staining properties.

(2) *Concentrated alcoholic solutions* are very convenient as stock preparations from which most of the usual anilin stains are made by proper dilution with distilled water. The selected anilin color is dissolved in absolute or 95-per-cent. alcohol to saturation, the precaution being generally taken to see that any excess of the dye which may be present lies at the bottom of the flask. In general one can estimate that from 20 to 25 gm. of the dye will be needed to every 100 c.c. of alcohol. Five or six drops of one of these concentrated solutions added to a small dish of distilled water affords a mixture suitable for staining films or even sections of tissue containing certain bacteria. Certain of the anilin stains in more or less general use are not, as a rule, employed in these concentrated alcoholic solutions. These are vesuvin, Bismarck brown, and anilin blue. In the event that these stains cannot be prepared each time in watery solution, Koch recommends that a concentrated solution made with equal parts of glycerin and water shall be kept on hand as a stock preparation. Fluorescein and eosin may be kept in solutions in absolute alcohol of the strength of 1 to 2 per cent.; the latter at the time of using may be further diluted with water until it yields a light rose-colored solution. For special methods of staining, the eosin is used in stronger concentrations, the most common form being an aqueous 5-per-cent. solution.

(3) *Solutions of Methylene Blue.*—This stain is prepared in a variety of ways:

(*a*) *Koch's Alkaline Solution of Methylene Blue:*

Concentrated alcohol solution of methylene blue,	1 c.c.
Distilled water,	200 "
Ten-per-cent. caustic-potash solution,	0.2 c.c.

(*b*) *Loeffler's Alkaline Methylene Blue:*

Concentrated alcoholic solution of methylene blue,	30 c.c.
Solution of potassium hydrate 1 : 10,000,	100 "

(*c*) *Unna's Alkaline Methylene Blue:*

Methylene blue,	1 part.
Carbonate of potassium,	1 "
Water,	100 parts.

For staining bacteria either in films or in sections this mixture is diluted in the proportion of 1:10 or even more.

(*d*) *Unna's Polychrome Methylene Blue:*

The polychrome methylene-blue solution recommended by Unna is an old alkaline solution made according to formula (*c*), in which, as a result of an oxidation process, methyl violet or later methyl red has been formed. Several months are required for the transformation of one into the other, but the ripened solution may be obtained from dealers. In using it the same dilution is employed as in the previous formula.

(*e*) *Kühne's Methylene-Blue Solution:*

Methylene blue,	1.5 gm.
Absolute alcohol,	10 c.c.
Five-per-cent. carbolic-acid solution in water,	100 "

Alcohol is poured over the methylene blue in a mortar, and the carbolic-acid solution is then gradually added with gentle stirring until solution is effected.

(*f*) Borax may be substituted for the carbolic-acid solution. The formula recommended by Sahli consists of:

Distilled water,	40 parts.
Saturated watery solution of methylene blue,	24 "
Five-per-cent. solution of borax,	16 "

This mixture is filtered after having been permitted to stand for one day.

(*g*) *Gabbett's methylene-blue solution* is used in connection with fuchsin as a counter stain in examinations for the presence of the tubercle bacillus. It consists of:

Methylene blue,	2 parts.
Sulphuric acid,	25 "
Water,	75 "

The mixture is allowed to stand for a day or two, the clear fluid being decanted from any precipitate which may form. It is durable and a rapid decolorizer as well as a contrast stain, more especially for the examination of sputum suspected of containing the tubercle bacillus.

(4) *Solutions of Gentian Violet and Fuchsin.*—These dyes can be used in simple aqueous solution, but are usually employed in solu-

tions containing a mordant. The mordants most commonly employed consist of pure anilin (anilin oil) and of carbolic acid.

(*a*) *Ehrlich's Anilin Water.*—Pure anilin oil is added in excess to distilled water, and shaken vigorously for one minute. About 5 c.c. of anilin oil is to be mixed with 100 c.c. of water of which 3 to 4 per cent. is dissolved. After permitting this mixture to stand for about five minutes, it is filtered through previously moistened filter paper. The filtrate should be perfectly clear, and is used in place of water as a menstruum. Inasmuch as the saturated solution of anilin oil tends to spoil, and can be very quickly prepared, it is advisable to make it fresh for each operation. When, however, it is desirable to keep a stock on hand, from 5 to 10 per cent. of alcohol is added to the finished solution (B. Fraenkel). A solution that need not be filtered may also be prepared by dissolving 3 c.c. of anilin oil in 7 c.c. of alcohol, to which is added 90 c.c. of distilled water.

As suggested by Ehrlich, it is generally convenient to prepare the staining-solutions consisting of fuchsin or gentian violet or mythyl violet, by simply adding a sufficient quantity of the saturated alcoholic solution of these dyes to the anilin-oil water until a perceptible opalescence appears in the fluid. This is indicative of saturation and the stain is now ready for use.

(*b*) *Weigert-Koch Anilin-Oil Fuchsin or Gentian Violet:*

Anilin water,	100 c.c.
Concentrated alcoholic solution of fuchsin or gentian violet,	11 "
Absolute alcohol,	10 "

This solution can be kept for ten to twelve days, after which it gradually loses its staining-power.

(*c*) *Stirling's Gentian Violet:*

Anilin oil,	2 c.c.
Absolute alcohol,	10 "
Water,	88 "

The anilin oil is to be dissolved in the alcohol, and the solution added to the water. In this fluid, which should be clear and free from excess of anilin oil, 5 gm. of gentian violet is dissolved. The mixture is filtered, and the stain is now ready for use. It is durable, and stains intensely and rapidly. For the purpose of staining tissues, it usually requires dilution.

Several mordants have been from time to time proposed in place of the anilin oil: toluidin (B. Fraenkel), turpentine (Prior), carbolic acid (Ziehl), ammonia (Weigert), borax (Sahli), carbonate of am-

monium (Kühne), and thymol (Brieger and Klemperer). Of these various chemicals experience has shown carbolic acid to be the one most generally applicable. The carbolized solution of fuchsin as recommended by Neelsen has found the most extensive use.

(*d*) *Neelsen's Carbol-Fuchsin:*

Fuchsin,	1 gm.
Absolute alcohol,	10 c.c.
Five-per-cent. watery carbolic-acid solution,	100 "

(*e*) *Czaplewski's Carbol-Gentian-Violet.*—With the exception that 2½ per cent. carbolic-acid solution in water is substituted for the solution of anilin oil, the procedures as given by Ehrlich are to be carried out. The precaution to be taken is to see that a saturated alcoholic solution of gentian violet is employed. This can be made by dissolving 7 gm. of gentian violet in 100 c.c. of alcohol.

(*f*) *Czaplewski's Carbol-Fuchsin.*—One gram of fuchsin is powdered and dissolved in 5 c.c. of liquefied carbolic acid. After the crystals are dissolved, 50 c.c. of pure glycerin is gradually incorporated with constant trituration. Thy glycerin is to be followed by the addition of 100 c.c. of distilled water. The resulting solution represents the stock mixture which keeps indefinitely, and can be employed without filtration. It is capable of being diluted to any extent with water without the occurrence of precipitation. In its undiluted form it is adapted for staining the tubercle bacillus; but for ordinary preparations and for counter-staining in the use of Gram's method, this concentrated solution is diluted with from four to ten parts of distilled water. The dilute solutions also can be kept for a long time without losing their properties.

(*g*) *Carbol-Thionin Blue.*—The stock solution consists of 1 gm. of thionin blue dissolved in 100 c.c. of a 1:40 carbolic-acid solution. This mixture, after being diluted in the proportion of 1 to 3 with water and then filtered, is ready for use. This staining-agent is suitable both for cover-glass preparations and for sections. For the former it requires no subsequent decolorization, but sections of tissues after an immersion of five minutes in the solution should be thoroughly washed in water, and decolorized with very weak (1:1,000) solutions of acetic acid. The dehydration is accomplished with absolute alcohol or anilin oil. Thionin blue stains more intensely than methylene blue, and gives equally good differentiation. It is recommended especially for staining the typhoid and glanders bacillus in sections.

Weigert's and Gram's Methods.—These methods, which differ only in minor points, and are applicable to the same purposes, serve for

the staining of cover-glass preparations containing bacteria, and also of bacteria in tissues. As has already been mentioned, while all bacteria are stainable by the dyes employed in these methods, only certain kinds are capable of retaining the stain in the subsequent treatment, to which the preparations are exposed. This fact is advantageous for two reasons. In the first place, we can arrive at a separation of the pathogenic bacteria into two great groups according as they do or do not remain stained after treatment by these methods, which can, therefore, be to a certain extent utilized for the differentiation of bacterial species or forms. In the next place, their employment permits us better to distinguish bacteria occurring in tissues, since, if the precaution is taken to counter-stain the cell nuclei, it is often possible to bring out the sharpest contrast between the tissue elements and the included bacteria. In both Gram's and Weigert's method the essential feature is the treatment of the tissue or film, after staining, with a solution of iodine composed as follows:

Iodine,	1 part.
Potassium iodide,	2 parts.
Distilled water,	300 "

Although the principle is the same, the procedure is slightly different according as these methods are applied to film preparations or sections. In the case of film preparations the steps are as follows:

(1) Stain in anilin-oil gentian violet five minutes; wash in water.

(2) Drop on the iodine solution and allow it to remain from half a minute to two minutes; wash in water.

(3) Decolorize in alcohol until almost all of the dye has been removed (Gram's method); or in anilin oil until the same degree of decolorization is obtained (Weigert's method). If the latter method is employed, the excess of anilin oil is removed with xylol; dry.

(4) Mount in xylol balsam.

For tissues the steps are somewhat different:

(1) Stain the sections lightly in carmine; transfer to water, and then to the slide where the section is straightened out and blotted with filter paper; it must not be completely dried.

(2) Drop on gentian-violet anilin oil. Allow to remain three to five minutes. Blot.

(3) Treat with iodine solution from half a minute to three minutes until a purplish-black color has been obtained. Blot.

(4) Decolorize and dehydrate in alcohol according to Gram's, or in anilin oil according to Weigert's method. If the latter is employed, the excess of oil is again removed by means of xylol. By the time that the decolorization is completed the sections are also

usually dehydrated. In Gram's method the alcohol is followed by clove oil or xylol to clarify before putting in the balsam.

(5) Add balsam and cover slip.

In the staining of films by this method, only the microorganisms are stained, as a rule, although if tissue cells chance to be present their nuclei may retain a part of the color, especially if alcohol has been used as the decolorizing agent. In tissues, on the other hand, the nuclei of cells appear red and any bacteria present capable of retaining the stain exhibit a sharp contrast in violet.

Czaplewski has recommended the carbol-glycerin-gentian-violet as the chief stain and the carbol-glycerin-fuchsin as the counter-stain in the employment of these methods.

As has been pointed out especially by Czaplewski,[29] these methods as applied to the differentiation of bacterial species, according as they do or do not stain by them, afford a relative and not an absolute mode of distinction. Whether or not the bacteria remain stained or become decolorized usually depends in part upon the length of time of action of the staining and the fixing solutions (iodine), and the length of immersion in the alcohol or anilin baths. Another difference observed depends upon the age of the cultures. Only young cultures show a uniform homogeneous staining, whereas, with increasing age of the culture, the number of homogeneously stained individuals diminishes proportionately. In the determination, then, of the fact whether or not an organism stains according to these methods, it is necessary to take into consideration not only the age of the culture, but more especially the length of time during which it has been exposed to the decolorizing solutions. Again, in the staining of sections, and sometimes even of film preparations, the color adheres to the tissues or to the film with great persistency, and is given up very slowly indeed to the decolorizing agents. It has been found by Czaplewski that in the application of Gram's method a momentary application of a drop of anilin oil to the specimen facilitates the subsequent decolorization by alcohol, and vice versa that a similar application of alcohol in carrying out Weigert's stain will assist the subsequent bleaching in anilin oil.

The Staining of Spores.

When bacilli containing spores are stained in the ordinary watery solutions of anilin dyes, the spores remain uncolored. But although they are more refractory than the vegetative protoplasm of the bacilli, once having been stained the spores retain the dye with greater persistency than the bacilli proper. Several methods are recommended for staining these varieties of organisms.

(*a*) *Abbott's method.* A cover-glass preparation is stained deeply with Loeffler's methylene blue, and carefully heated until the staining solution boils for a period of about one minute. The boiling should be discontinuous, which is effected by alternately removing and replacing the film in the flame. The film is then washed in water and dipped five or six times in alcohol containing from 0.2 to 0.3 per cent. of hydrochloric acid. After being again rinsed, it is stained for from eight to ten seconds in anilin-water-fuchsin solution (Koch-Ehrlich), and finally is again washed in water. By this method the spores appear of a blue color and the body of the bacterial cells of a red color.

(*b*) The cover slip is floated with the impregnated side downwards, upon the surface of a freshly prepared anilin-water solution of fuchsin contained in a watch crystal. The watch crystal is held about 2 cm. above the flame of a Bunsen burner, care being taken that the flame touches only the centre of the crystal. After being left for a few seconds at this level, the crystal is elevated gradually until it is about 6–8 cm. above the flame, when it is slowly lowered, this up-and-down movement being continued until the fluid boils. As soon as boiling is established, the crystal is held aside for a minute or two, after which the process of heating is repeated until it has been carried out for five or six consecutive exposures. After the last boiling, the preparation is allowed to remain in the fluid for about five minutes, and is then transferred directly to a second watch crystal containing the decolorizing solution, which consists of three per cent. hydrochloric acid in absolute alcohol. The film is floated in a similar way upon the surface of this solution, the vessel being gently tilted from side to side for a period of about one minute. It is then removed, washed in water, and stained in the ordinary way with the alkaline methylene-blue solution. The spores will be found to have assumed a red, and the body of the bacterial cell a blue color.

(*c*) *Moeller's method.* After being prepared in the usual way, the film is placed for two minutes in chloroform, and next for a period varying from half a minute to two minutes in a five-per-cent. solution of chromic acid; it is then washed off with water and stained in carbol fuchsin. The staining is carried out directly over the flame. Carbol fuchsin having first been placed upon the cover slip, the latter is held in the flame until boiling takes place, and then for a minute some distance above the flame. The decolorization is accomplished by means of five-per-cent. solution of sulphuric acid. The excess of acid is removed by washing in water, and the film is finally stained for a period of thirty seconds in the alkaline methylene-blue solution. The spores appear red upon a blue ground.

Staining of Flagella.

The method originally introduced by Loeffler still suffices for the demonstration in a very satisfactory way of the flagella in many bacteria. Since his discovery numerous modifications, and indeed new principles, have been introduced into the staining of these appendages, and the methods employed for this purpose are quite numerous.

In the staining of flagella great care is necessary if anything approaching success is to be obtained. In the first place the cover glasses must be scrupulously clean and free from all traces of grease. In order to accomplish this, it is necessary to treat them with sulphuric acid and potassium bichromate, and afterwards with alcohol and ether and ether alone in order to remove the oil. After the immersion in the ethereal bath, it is best to permit them to become air dry instead of wiping them with a cloth. Young cultures are to be used. They should be taken from the surface of the growth upon agar-agar, the most favorable period being after twelve to eighteen hours' exposure to a temperature of 37° C. Several of these clean cover slips, from four to six, are arranged in a row upon a piece of white paper, and upon the centre of each is placed a small droplet of sterilized distilled water. These droplets are now inoculated in order by means of a platinum needle charged with a small amount of the growth from the agar surface. The needle is not recharged until the entire series has been inoculated, and in this way a successive dilution of the culture in the course of the several inoculations is obtained. The transference of the material to the droplets of water upon the films should be carried out gently, so as to avoid as much as possible any separation of the flagella from the bacterial cells, and the fluid is next gently spread out upon the surface and permitted to become air dry. If the cover slips have been properly cleansed, there will be little or no tendency for the coalescence of the film into droplets.

A second mode of procedure is to make a suitable dilution in a watch glass, containing sterilized water, and to prepare the films by placing a drop of this fluid upon the cover slip, and gently spreading it over the surface. In order that this latter method may give good results, it is necessary that the original dilution should be of exactly the right degree, whereas in the former procedure, in which successive dilutions are employed, this difficulty is obviated, since it will generally happen that of the four or six cover slips prepared two or four contain organisms in an appropriate state of dilution. For staining the following methods may be mentioned:

(*a*) *Loeffler's method.* Two solutions are required.

(1) Twenty per cent. tannic acid in water, ... 10 c.c.
Cold saturated solution of ferrous sulphate, . . . 5 "
Saturated alcoholic or watery solution of fuchsin, . . 1 "
This solution represents the mordant.

(2) One-per-cent. solution of caustic soda and a solution of sulphuric acid of such a strength that 1 c.c. will be exactly neutralized by 1 c.c. of the soda solution. Mix thoroughly.

Different species of bacteria require different quantities of acid and alkali in order that the mordant shall properly affect them. The acid and alkali are added to the mordant before the film is treated with this agent. For demonstrating the flagella of typhoid bacilli it has been found that 10 c.c. of the mordant require at least seven drops or even a larger quantity of the sodic hydrate solution. The first tests had better be made with seven drops, and if they are unsuccessful, the alkali is increased drop by drop until the proper staining is effected. The details of the process are as follows: To the film add the requisite quantity of mordant containing the alkaline addition. Hold well above the flame for one to one and one-half minutes, permitting the mordant to steam, but avoiding boiling. Wash thoroughly in water and then treat with carbol-fuchsin or anilin-oil gentian violet in the usual way, exposing again to the action of the flame for about the same length of time allowed for the heating of the mordant. Wash again thoroughly in water, dry, mount in balsam, and examine. It is advisable to scrutinize the whole or a large part of the field carefully before deciding that the specimen is unstained. For the demonstration of the B. coli communis a larger quantity of alkali is required. To 10 c.c. of the mordant it is advisable to begin with the addition of at least nine drops of the alkaline solution and then gradually increase drop by drop until the desired staining is obtained. Spirilla can, as a rule, be successfully stained with the unadjusted mordant; sometimes the addition of acid is called for.

(*b*) *Bunge's method.*

Solution (1):

Saturated solution of tannic acid, ... 3 parts.
Solution of liquor ferri sesquichlor. 1 : 20, . . . 1 part.

Solution (2):

Mixture (1), ... 10 parts.
Concentrated watery solution of fuchsin, . . . 1 part.

The resulting mixture is to be exposed to the air for a period varying from several days to several weeks, the older solutions staining more successfully than the fresher ones. The preliminary steps in

the preparation of the cover slips are identical with those already given. The films, after flaming, are treated with the filtered mordant, which is allowed to act in the cold for about five minutes, after which it is slightly warmed. The film is rinsed thoroughly in water, dried, and then stained with carbol-fuchsin.

(*c*) *Van Ermengem's method.*

Solution (1):

Two-per-cent. solution of osmic acid,	1 part.
Ten to twenty-five per cent. solution of tannin,	2 parts.

The films are to be kept in this fluid for one hour at room temperature; or they are heated in it over the flame until steam rises and then allowed to remain in the hot solution for at least five minutes. They are now removed, washed with distilled water and then with absolute alcohol, remaining in the latter from three to four minutes, when they are again rinsed in distilled water.

Solution (2):

0.25–0.5 solution of silver nitrate in distilled water.

The films having been treated with solution (1) are now placed in solution (2), where they are allowed to remain for a few seconds. Without washing they are transferred to solution (3), consisting of:

Tannin,	3.0 gm.
Gallic acid,	5.0 "
Fused potassium acetate,	10.0 "
Distilled water,	350 c.c.

Keep the film in this solution for a few seconds. Remove, pass again into solution (2) and then into (3). These transfers are to be made back and forth until the film assumes a rich brown color; avoid the appearance of blackness. A few precautions are to be observed. In the first place, the silver bath is to be changed as often as a precipitate forms within it. Secondly, a fresh supply of solution (3) for each preparation is also advantageous. By this method, when successfully carried out, admirable preparations of flagella of many organisms can be obtained. It is now used more extensively than Loeffler's method or its modifications.

Inoculation of Animals.

The use of the lower animals for the experimental demonstration of the pathogenicity of microorganisms has several purposes. In the first place it is possible with some organisms to reproduce in animals experimentally the pathological condition present in the natural dis-

ease with which the particular microorganism has been associated, and from the lesions of which it has been obtained. Such a demonstration affords a strong argument in support of the belief of the relation of any one particular microorganism to a given pathological process.

Pathogenicity in an organism is not synonymous with the production of typical lesions of disease by that organism. Certain animals succumb to inoculation with pathogenic organisms, and yet fail to yield either the symptoms characteristic of the disease from which such organisms have been obtained, or the pathological lesions which in the original animal brought about the clinical symptoms. Thus the typhoid bacillus is pathogenic for small animals, even causing their death, and yet at the same time it does not provoke in them the characteristic intestinal and other lesions with which it is associated in human beings. In the case of some organisms, however, by inoculation into animals we are able to reproduce the specific characteristics of the disease, and are also enabled to separate the large group of saprophytic or non-pathogenic from the smaller group of parasitic and pathogenic organisms. Microorganisms of a pathogenic character are susceptible of having this virulence greatly intensified, such an intensification being accomplished by successive passages through the animal body, which, along with other modifications, tends to promote their adaptation to a parasitic mode of existence at the expense of power for saprophytic propagation and vitality.

The inoculation of animals with microorganisms is also useful for the separation of combinations of species. In mixtures of pathogenic and non-pathogenic forms the animal can be made to serve for the isolation of the former, which alone are capable of surviving for a time in the animal body, or of bringing about its death, the saprophytes being almost immediately destroyed and disposed of. In general it may be said that when several species of microorganisms, of which more than one are pathogenic, are introduced into the animal body, they will be so modified in their capacities for continued development that only the most virulent will survive and be capable of recultivation from their new habitat.

Mode of Inoculation.

The mode of inoculation will vary according to the nature of the animal and the purposes to be achieved. In general a distinction is made between subcutaneous, intraserous, intravascular, and intracerebral inoculations. In the first the material for inoculation is carried beneath the skin and deposited in the subcutaneous tissue;

in the second it is introduced into one of the great serous cavities; in the third the injection is made directly into the circulation, usually into one of the veins; while in the last the inoculation is made, after trephining the skull, directly into the cerebral substance. Small animals, such as mice, rats, and guinea-pigs, are most successfully inoculated subcutaneously or into the serous cavities; while larger animals, for example rabbits and dogs, may be used for any of the methods enumerated.

Sources of the Material.—The material chosen for inoculation may be derived from pure cultures, or from tissues, secretions, or excretions of the animal body, or directly from nature.

If it is intended to observe the action of any given microorganism, care must be exercised to avoid contamination of the inoculated material. Indeed, even if mixtures of organisms are to be injected, equal care should be exercised in order that additional extraneous organisms shall not find their way into the body at the time of the inoculation. For this reason all of the instruments used in the experiments should be sterilized, and the tissues of the animal should be aseptically prepared. The hairy parts of animals are to be shaved, and the part exposed is to be cleansed with bichloride of mercury solution, and afterwards with alcohol and ether. If an incision is necessary for the purpose of introducing the inoculated material the wound should be closed with a dressing of celloidin, etc.

Especial problems are presented according to the animal or the site of inoculation chosen. Injections into the peritoneum and the pleuræ require care in order to avoid injuring the viscera contained within these cavities. Special syringes have been constructed for the purpose of facilitating intraserous inoculations. These, however, can generally be dispensed with. Unless there be reason to the contrary in making injections into the pleural cavity the right side should be selected rather than the left in order to avoid injury to the pericardium and heart.

Thus far intracerebral inoculations have been employed, first, in order to intensify the effects of certain pathogenic microorganisms (B. tetani; B. diphtheriæ), and second, for the purpose of studying the direct effect of these organisms or their poisons upon the ganglionic nerve cells. The amount of fluid, according to Durham, which can be injected with safety into the brain of rabbits varies from 0.5 to 1.0 c.c., while in guinea-pigs it should not exceed 0.3 c.c.

PATHOGENIC BACTERIA.*

Coccaceæ.

Spherical, sometimes slightly elongated microorganisms occurring singly or grouped in pairs, chains, or grape-like clusters. Growth is usually obtained upon the ordinary culture media; exceptionally special nutritive mixtures are required (gonococcus). Staining is readily accomplished with the anilin dyes, but the behavior with Gram's or Weigert's methods is variable. The most general characteristic belonging to this group is the faculty of causing suppuration.

Staphylococci.

Spherical cells tending to form grape-like clusters, more rarely appearing as diplococci, or in short irregular chains.

Staphylococcus Pyogenes Aureus. *Morphology and Cultural Properties.*—This organism was originally described by Ogston, and was first cultivated artificially by Rosenbach, Krause, and Passett. The individuals average 0.87 μ in diameter. With Gram's method the color is usually retained, or is liberated only with difficulty and after a long immersion in alcohol.

The organism grows upon all ordinary culture media, and possesses the property of peptonizing gelatin. At first the growth may be white in color, but sooner or later a golden-yellow pigment is developed. The rapidity of development and intensity of color of the pigment depends in part upon the culture medium. Potatoes afford a soil especially favorable for the development of the pigment.

Fig. 5. — Staphylococcus Pyogenes Aureus. (Rosenbach.)

Gelatin.—On gelatin plates a growth develops at the end of the second day at room temperature into point-like colonies, which under low powers of the microscope appear as faintly brownish, smooth, and spherical dots. The centre of the colonies often presents a deeper color than the periphery. The colonies reach the surface of the medium about the third day, at which time the pigment may also begin to appear.

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* References to the literature are only rarely given in the following pages. Acknowledgment in general is made to the works and publications mentioned at the end of this article. The general current literature on medical bacteriology has been fully used.

At this stage of development the gelatin begins to be liquefied at the periphery, and as a result the microscope gives a double contoured form to the colony, which need not exceed one millimetre in diameter. The liquefaction of the gelatin proceeds from this time on with considerable rapidity until, the superficial colonies developing close to one another, coalescence occurs and the medium eventually becomes entirely liquefied. Stab cultures at first show a white growth along the line of inoculation which soon causes liquefaction of the medium, and later pigmentation, the color appearing first in the upper layers of the growth with which oxygen comes immediately into contact. With total liquefaction of the gelatin the growth settles to the bottom as a golden-yellow mass, which under the microscope is seen to be composed of grape-like clusters.

Agar-agar.—The individual colonies reach about the same size as in the former medium, but because of the absence of liquefaction less characteristic appearances are developed than with gelatin media. The colonies may be somewhat more coarsely granulated and more opaque. The most characteristic appearance in this medium depends upon the development of the pigment. In stick cultures the growth takes place along the line of puncture. At first it is white, and only gradually, after some days, a yellow color makes its appearance from above downwards. Upon the surface of agar-agar slants the growth at first develops along the line of inoculation, but tends to spread to either side along the moist agar surface. The edges of the growth are irregular, crenated, and slightly thickened. The pigmentation begins at this edge and proceeds towards the centre. No liquefaction occurs.

Potato.—On potato the growth, at first white, quickly takes on a pale yellow tinge. Later as it increases in thickness it becomes succulent in appearance, and develops a golden-yellow color. At first development takes place about the point of inoculation, but the growth gradually extends until it covers a considerable part of the surface of the inoculated substance.

Milk.—In plain milk coagulation takes place in from one to eight days in the thermostat. Litmus milk is first rendered acid and later is coagulated. The color of the litmus may be partially or completely discharged, but this decolorization is not permanent, since it depends upon the absorption of oxygen by the growing organisms. Agitation of the decolorized fluid so as to incorporate atmospheric oxygen brings about the restoration of the color and indicates the acid reaction. The acids formed are lactic, isobutyric, valerianic, and propionic, the first predominating.

Bouillon.—The growth is rapid and produces a uniform clouding of the medium. After some days a sediment appears, although the medium as a rule does not become entirely clear.

Pathogenicity.—In human beings this organism is the most common cause of suppuration. It has been found in acute abscesses, furuncles, phlegmons, anginas, suppurative processes in the middle ear, inflammations of the serous cavities, joints, and meninges, in osteomyelitic foci, upon the heart valves, in puerperal sepsis, etc. A case of pyæmia in association with an ulcer of the stomach was thought by Widal and Meslay to be due to the localization of staphylococci in the mucous membrane. The writer observed an instance of localization of the same organism from endocarditic vegetations within the mucous membrane of the stomach, with the production of a gastric ulcer. Cryptogenetic infections have also been referred to this organism. From localized foci the organism may penetrate into the lymphatics, bringing about an extension of the process into the neighboring structures, while at other times it may pass into the circulation, through either the lymphatic vessels or the blood-vessels, and set up a general infection, which may be of the nature either of a *septicæmia* (bacteræmia), in which no secondary microscopic foci of localization occur, or of a *pyæmia*, in which abscesses in one or several organs may exist. The microorganism can be cultivated from localized foci and from the blood during life. Not all human beings are equally susceptible to infection with Staphylococcus pyogenes aureus. Marked individual peculiarities are known to exist, and the previous occurrence of constitutional diseases undoubtedly predisposes certain individuals to its pathogenic action. Certain cachectic and constitutional disorders, such as diabetes, Addison's disease, tumors, and chronic cardiac, hepatic, and renal diseases, should be mentioned as especially favoring infections (Flexner). Garré showed that, by rubbing cultures of Staphylococcus aureus (obtained from an osteomyelitic focus) into the unbroken skin of the arm the organisms which found their way into the excretory ducts of the epidermal glands would set up an ordinary furunculosis.

The lower animals are susceptible to infection with the staphylococcus, but the effects of inoculation differ according to the kind of animal, the mode of application, and the virulence of the organism. Those thus far experimented upon have all proved to be less susceptible than human beings, and relatively large quantities of the organism are required to produce infection in them. In rabbits, guinea-pigs, and mice subcutaneous inoculations are either ineffectual or are followed only by abscess formation with rapid healing. Inoculation into the anterior chamber of the eye of rabbits (Th. Leber) is

followed by local development, production of hypopyon, perforation of the sclera, but eventual healing. Intraperitoneal injections are usually more serious. Some animals succumb at the end of from two to ten days, and lesions indicative of an acute peritonitis are found. Not infrequently, however, healthy animals are capable of resisting large quantities injected into the peritoneum. The intravenous inoculation reproduces the morbid conditions found in the general infection in man. In the rabbit, death may follow within twenty-four or forty-eight hours, and great numbers of staphylococci may be found in the blood and the organs. At other times when the fatal result has been delayed for several days, in addition to the general distribution of the organisms, pyæmic abscesses are found in the kidneys, heart muscle, voluntary muscles, and less frequently in the central nervous system. An unusual localization is in the serosa of the heart valves, with consequent ulceration. Endocarditis can be induced with certainty if prior to the injection, or immediately afterwards, the heart valves be mechanically injured. In young animals intravenous inoculation is followed not unusually by localization of the cocci in the medulla of bone, with a resulting osteomyelitis. Injury to bone in mature animals brings about a similar localization. More rarely the organisms attack by preference one of the serous membranes, producing an acute serositis.

Poison Production.—The Staphylococcus aureus elaborates in its growth a poison. The dead organisms introduced beneath the skin or into the interior chamber of the eye cause an emigration of leucocytes, purulent infiltration, and in the latter situation hypopyon formation. Sufficient amounts of the dead organisms when introduced into the peritoneal cavity of dogs and guinea-pigs cause death. This poison is known only by its effects. The production of suppuration depends upon a substance intimately associated with the protoplasm of the bacterial cell. As to the exact nature of the several chemical substances obtained from bouillon and peptone cultures of the organism, different investigators are not agreed. Phlogosin, a crystalline principle and highly pyogenic, isolated by Leber, fails to give the albumin reaction. It is probably not the most active poisonous principle of the organism.

Immunization.—Active immunity can be produced in various species of animals by the injection of increasing quantities of living cultures of staphylococcus. The same effect was produced by Reichel by means of injections of cultures killed by heat. The serum of animals which have been actively immunized, when injected into other animals, gives to the latter a passive immunity, and, according to Viquerat, the serum possesses in addition healing properties. Simi-

lar properties, according to this author, are found in the blood serum of human beings who have recovered from osteomyelitis.

STAPHYLOCOCCUS PYOGENES ALBUS.—This organism differs from the preceding chiefly through the absence of pigment production. It was found by Rosenbach in association with Staphylococcus aureus, and has since been obtained along with this organism and Streptococcus pyogenes, and also alone in suppurative foci. Excluding the formation of pigment Staphylococcus pyogenes albus agrees in its microscopic, cultural, and pathogenic properties with Staphylococcus aureus. Owing to the fact that the latter in its growth upon certain media and under certain circumstances appears white, and only slowly changes to yellow, the two organisms are very easily confounded, unless care be exercised to bring out by special cultural methods the possible pigment production of the aureus. *Staphylococcus epidermidis albus* is another variety, which has been found by Welch to be a regular inhabitant of the epidermis, lying deeper than can be reached by disinfection of the surface of the skin. Its growth is somewhat slower, its liquefaction of gelatin and coagulation of milk somewhat less rapid than in the case of Staphylococcus albus.

STAPHYLOCOCCUS PYOGENES CITREUS.—This was first cultivated by Passet in 1885 from abscesses. It differs from the aureus and albus only by forming lemon-yellow pigment in the presence of free oxygen. It possesses pathogenic effects similar to those of the aureus and albus, though it often shows less virulence than the aureus. It is found less frequently than the staphylococci which have just been considered.

STAPHYLOCOCCUS CEREUS ALBUS was cultivated from two abscesses by Passet in 1885, but its pathogenic power was not considered by Passet and Flügge to have been demonstrated. Levy has found this coccus in pure culture, in abscesses and other inflammations, and produced with it suppuration by inoculation into the rabbit's eye. It does not liquefy gelatin and forms no pigment.

STAPHYLOCOCCUS CEREUS FLAVUS was isolated by Passet in pure culture from chronic suppurative periostitis. It does not appear to have been found again in abscesses. Passet was unable to cause abscesses by inoculating it into animals. It differs from Staphylococcus cereus albus only in forming a lemon-yellow pigment.

MICROCOCCUS PYOGENES TENUIS was found by Rosenbach in some ten per cent. of cases of unopened abscesses studied by him. The cocci are irregular in size, somewhat larger than staphylococci, and in contrast with these show little tendency to the formation of clusters. The stained preparations often show two dark polar bodies separated by a pale intermediate substance. Cultures upon agar appear as

thin, almost hyaline growths, while in stabs they present a thicker and more opaque layer. It is believed by many that this organism is probably identical with Micrococcus lanceolatus.

Micrococcus du Clou de Biskra.—Biskra button or Aleppo boil is an endemic disease occurring in Aleppo, Bagdad, Biskra, and Tunis. It appears in the form of swellings of the face and extremities, which run a somewhat chronic course, so that within a year they rupture and finally cicatrize. Duclaux found in the blood of a patient with this affection micrococci which are less than 1 μ in diameter, and occur as diplococci, or in zooglœa forms. The organism grows in neutralized calf's bouillon.

Subcutaneous injections into the rabbit produce extensive inflammation, attended with death of the inflamed part, but finally healing takes place. Intravenous inoculations are followed by death in about sixteen hours, and in these cases pericarditis and pleuritis, hemorrhagic infarction of the lungs, etc., have been described. Smaller doses injected intravenously produce numerous ulcerating nodules over the body. A similar micrococcus has been described also by Heydenreich and Chantemesse in the nodules of this affection.

Micrococcus Zymogenes. — MacCallum and Hastings have described a small, somewhat elongated diplococcus occurring sometimes in chains of four, but generally in pairs, which stain by the ordinary methods as well as those of Gram and Weigert.

In its growth upon agar-agar it resembles Streptococcus or Micrococcus lanceolatus. Upon potato growth is relatively slow, but after two or three days a dry whitish or tawny layer appears. Litmus milk is at first decolorized, but within forty-eight hours coagulation and acidification take place. The clotted mass undergoes rapid peptonization. Coagulated blood serum and gelatin are also liquefied. The organism is hardy and can be recultivated from tubes several weeks old. Its thermal death point ranges from 60° to 65° C., with an exposure of five minutes.

Pathogenicity.—The organism was originally isolated from a case of endocarditis in a man. Mice and rabbits die after inoculations made either subcutaneously or into the serous cavities. After injury of the heart valves endocarditis is developed. An instance of localization upon the uninjured valves of the rabbit is reported. Dead cultures and the filtrates from cultures preserve the casein-coagulating and peptonizing property.

Streptococci.

Spherical Cells Tending to Appear in Chains and Rarely as Diplococci.

STREPTOCOCCUS PYOGENES; STREPTOCOCCUS ERYSIPELATOSUS.—Observed by Ogston in pus in 1881. Cultivated by Fehleisen from erysipelas in 1883, and by Sternberg from pus in 1884. The streptococcus of erysipelas does not differ in morphology or structural characteristics from Streptococcus pyogenes. The same pathogenic effects may be produced by each in animals and in man, so that according to Welch, the weight of evidence is in favor of the identity of the two organisms, although opinions are still divided on this question.

FIG. 6. — Streptococci Chains. Highly magnified. (Lehmann and Neumann.)

Morphology and Cultural Properties.—The cocci are spherical without independent motility, averaging 1 μ in diameter. They are usually slightly larger than staphylococcus, and stain with the ordinary fluids and by Gram's and Weigert's methods. Characteristic is their appearance in chains which are of variable length, and consist of eight, ten, twenty, or more members. By foldings and convolutions of the chains larger masses may be formed. Occasionally the organism appears in the form of a diplococcus. In the course of the chains there are sometimes developed individual organisms larger than the others (Hueppe's arthrospores). These are especially abundant in old cultures, and probably are to be regarded as involution forms.

Gelatin.—The streptococcus grows in gelatin plates as very small, point-like colonies, which when they reach the surface are elevated slightly above it, and have a diameter of about half a millimetre. In the stabs the growth is rarely continuous, but more often appears in the form of isolated colonies which scarcely exceed a pin point in size. After many days the gelatin colonies still preserve their original appearance, although perhaps increasing slightly in size and opacity, and only very exceptionally cause liquefaction of the medium (v. Lingelsheim, Escherith). Under low powers of the microscope the young colonies appear as round, more rarely oval, yellowish spots with regular contours and a finely granular surface. Somewhat later they appear darker, almost brown in color, and the edge of the colonies may be slightly interrupted and roughened from the outgrowth of chains at the side. Stab cultures usually show individualized colonies along the line of puncture.

Agar-agar, etc.—Upon agar-agar plates the individual colonies

grow rather larger and are somewhat more spreading and opaque. The slant growths upon gelatin or agar appear either in the form of individual colonies, or as a uniform, veil-like membrane, semitranslucent in appearance. On blood serum the growth is similar to that upon agar. Upon potato it is usually invisible.

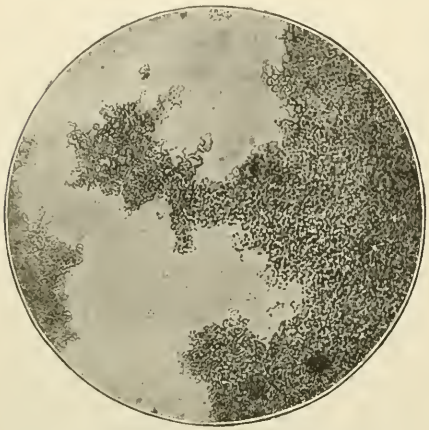

Fig. 7.—Agar-agar Culture of Streptococcus Pyogenes. × 100. (Heim.)

Bouillon.—In bouillon remarkable differences are encountered. Some races produce a clouding; others grow upon the sides and bottom of the tube, leaving the fluid throughout quite clear. The extent of growth and of sedimentation is variable, being very slight in some specimens; in others considerable acid is usually produced. Bouillon cultures are especially well adapted for the study of the normal morphology of the organism. According to its mode of growth in this medium varieties have been distinguished, such as Streptococcus conglomeratus, involutus, brevis, longus, etc. These differences do not indicate specific distinctions.

Milk.—The action upon milk is also variable. This medium may or may not be coagulated. Acid production is usually marked.

Pigment is sometimes produced. The colors vary from yellowish-red to orange or blood-red. According to Pasquale pigment appears in cultures of typical Streptococci and also in varieties of Streptococcus lanceolatus.

Some cultures are short-lived, others may exist for several months. In dried pus the streptococcus may survive for from fourteen to thirty-six days. It is killed in ten minutes by exposure to 54° C. (Sternberg), in eight seconds by three-per-cent. carbolic-acid solution. For the purpose of keeping the culture alive in the laboratory, gelatin stab cultures transplanted every five days are best.

Pathogenicity.—Streptococci in human beings are concerned with the production of suppuration, erysipelas, phlegmons, puerperal and general septicæmias, lymphangitis, anginas, pneumonias, especially of the lobular type, bronchitis, enteritis, hepatic abscess, periostitis, otitis, meningitis, pyelonephritis, osteomyelitis, empyema, endocarditis, and various inflammations of the serous membranes. It occurs

frequently alone as the specific cause of the disease, while at other times it is found in combination with other organisms in the so-called mixed infections. One of its important rôles as a secondary invader is in the ulceration taking place in phthisical cavities. It also plays a prominent part in the production of the severe secondary and septicæmic infections in diphtheria and scarlet fever. It is a constant attendant of the so-called scarlatinal diphtheria. It has been proven to be the cause of infections in the lower animals, as, for example, the acute umbilical phlebitis of horses (Casper). Pre-existing chronic disease favors its development (Flexner). Fehleisen succeeded in producing erysipelas in man from cultures of the seventeenth generation of streptococci obtained from that disease. The experiment was made in persons who were suffering from inoperable malignant tumors. The artificial production of such an *erysipèle salutaire* in the treatment of malignant tumors has been followed up, in this country especially, by Coley, who has, however, replaced the injections of the living cultures of the streptococcus, by the toxin, in order to avoid the local and even general destructive effects to which the former often gave rise.

Streptococci obtained from different sources vary greatly in pathogenic properties. The virulence varies not only in degree, but in kind, so that a streptococcus endowed with the property of producing one kind of infection, as, for example, erysipelas, may not be qualified under ordinary conditions to produce another kind of infection, as, for example, abscess. Despite this fact, however, transformations in these degrees and qualities are so easily brought about that no distinctive classification on this basis is possible. Most of our experimental animals are not particularly susceptible to infection with streptococci. White mice and white rabbits are best adapted for the study of their pathogenic properties. Inoculated subcutaneously into the rabbit's ear, virulent streptococci usually produce erysipelas from which the animal recovers. Very virulent cultures injected into the peritoneal cavity produce rapidly fatal septicæmia or local inflammations, and the mouse may die in a longer or shorter period from the effects of the inoculation without the presence of streptococci being detected at the autopsy. Virulent streptococci injected into the peritoneal cavity of rabbits produce, even in small doses, fatal peritonitis (Wallgren).

The effects upon the organs have been studied by Flexner and Homén and his pupils. The degenerations are produced both by the living organisms and the filtrates (toxin). Streptococci injected into nerve tissues spread through the lymphatic spaces centrally and peripherally. They reach the spinal canal travelling preferably along

the posterior roots of spinal nerves, and then pass by way of the meninges and the sheaths of the vessels in the cord to the brain. The effect upon the nerves is degeneration (Homén and Laitinen). Von Borndorff found after intravenous injections in rabbits that the cocci appeared inconstantly in the urine, but not until nine and one-half hours after the inoculation and apparently only after damage has been done to the kidneys.

Toxin.—The poison produced by the streptococci causes fever, constitutional symptoms, and death. The nature of this poison is not known. It is found in solution in the liquid media in which the cocci are grown. There is a relation between the virulence of the organism and the intensity of the poison. Marmorek has artificially increased enormously the virulence of streptococci by cultivations in human blood-serum bouillon, and by transference from animal to animal and recultivation.

Immunization.—Animals both large and small can be immunized against the streptococcus and its toxin, and the blood serum of animals thus actively immunized contains a principle which suffices to passively immunize other animals, and one that has been used therapeutically in streptococcus infections in human beings, although thus far with not very brilliant results. Immunization to Streptococci has given no basis for a systematic separation into different varieties.

Streptococcus equi (Schütz).—*Synonym*, S. coryzæ contagiosæ equorum (Eisenberg).

The cause of strangles (*Druse*) in the horse. First described by Schütz and later, independently, by Sand and Jensen and Poels. Schütz failed to cultivate the organism upon agar and gelatin, Poels succeeded with gelatin but not with agar, while Sand and Jensen obtained positive results with the latter, although it is acknowledged that for some unknown reason the organism sometimes fails to grow upon agar media.

Morphology and Cultural Properties.—As it occurs in the animal body, the streptococcus appears in long chains which sometimes extend across the field of the microscope. The forms vary somewhat with the stage of development: immediately after division of the cells, the greatest diameter is at right angles to the cells, but as the cocci grow older they gradually assume the spherical form. The morphology of the S. equi is not to be distinguished from the S. pyogenes. In cultures the former grows less easily upon gelatin; the staining properties of the organisms and many of their pathogenic effects are identical.

Pathogenicity.—The constant association of this organism with

strangles in the horse speaks for its pathogenic properties. It is regarded by Jensen as the specific cause of the disease. The organism is found in the catarrhal secretion and in the associated abscesses in the lymphatic glands and larynx. Sand and Jensen made inoculation experiments upon horses. Simple injection of cultures into the nostrils is without effect. Rubbing of cultures upon the nasal mucous membrane produces typical clinical symptoms, including the abscess formation of the disease. One such experimented animal succumbed to metastatic abscesses in the internal organs. In the similar abscesses of the natural disease S. equi has been found, as well as in thrombi upon the heart's valves (Zschokki and Jensen). The organism is carried from the mother to the fœtus (Nocard), in which cases abscesses are found in the lungs, spleen, kidneys, etc., of the latter. Evidences of pyæmia in the mother were wanting. The exanthematous cutaneous eruption, which appears late in the course of the disease, has been shown by Joly and Leclainche to be caused by special localization of the streptococcus.

Mice succumb regularly to inoculation; rabbits only after large quantities of bouillon cultures have been injected directly into the blood, while subcutaneous inoculation produces erysipelatous reddening of the skin only. Guinea-pigs are immune.

Immunity.—Friedberger and Fröhner state that after recovery from strangles there is an immunity that lasts at least a couple of years. Nocard and Leclainche have denied that such immunity is afforded by the natural disease. Sand and Jensen inoculated two horses intravenously without appreciable result, and later applied virulent culture to the nasal mucous membrane, but the animals remained well. Two horses that had recovered from the disease, produced by similar applications, were afterwards treated with pus from a submaxillary abscess and failed to react, while the control animal developed the disease. Hence they conclude that both natural and experimental infection gives immunity.

Diplococci.

Spherical, slightly elongated or flattened cells, appearing chiefly in pairs, sometimes in tetrads, more rarely in short chains.

Micrococcus Lanceolatus. *Synonyms.*—Diplococcus pneumoniæ, Diplococcus lanceolatus, Pneumococcus (Fraenkel and Weichselbaum), Diplococcus lanceolatus capsulatus, Micrococcus of sputum septicæmia, Micrococcus pneumoniæ cruposæ, Streptococcus lanceolatus. Discovered by Sternberg in his own saliva in 1880, and demonstrated to be the cause of lobar pneumonia by Fraenkel and Weichselbaum in 1886. The organisms described under these names

appear often to be closely related to the group of short streptococci (S. brevis).

Morphology and Cultural Properties.—The Micrococcus lanceolatus usually appears in the form of capsulated, lancet-shaped spheres united in pairs and short chains, more rarely in longer chains. It stains with the ordinary dyes, and by Weigert's and Gram's methods. It grows best at the body temperature, but may grow at from 18° to 22° C. It is capable of cultivation on all ordinary alkaline media, but is susceptible to slight variations in composition of the cultural media. The capsule of the organism is best demonstrated in the pneumonic exudate of croupous pneumonia, as it appears in the sputum and in the blood and organs of animals that have succumbed to inoculation. The lanceolate form is given by the sharpening of the ends, which usually are turned away from each other, but sometimes are in juxtaposition.

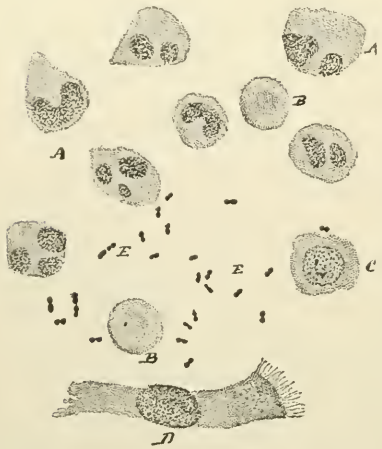

FIG. 8.—Diplococcus Lanceolatus in Pneumonic Sputum. *A A*, Leucocytes; *B B*, erythrocytes; *C*, epithelial cell; *D*, ciliated epithelium; *E E*, diplococci lanceolati. × 1,000. (Tuttle.)

Agar-agar.—The plate cultures in agar-agar resemble greatly the growth of Streptococcus pyogenes. The colonies are small, those in the depth remaining pin-point in size, whereas those which come to the surface spread slightly, reaching perhaps half a millimetre in diameter, and show a film-like appearance. Under the microscope they are slightly granular. The addition of blood or glucose or glycerin to the agar improves it as a culture medium and enables the colonies to reach a somewhat larger size. The growth upon the agar slant is also very similar to that of streptococcus, being, however, somewhat less profuse than is seen in many examples of that organism.

Gelatin and Guarnieri's Medium.—The greatest resemblance in growth to that of the streptococcus is found in gelatin incubated at temperatures from 22° to 24° C. Liquefaction does not take place. Owing to the difficulty of cultivation of this organism special kinds of media have been introduced. That recommended by Guarnieri (see page 584) is perhaps the best. The organism grown in this medium will sometimes show capsules.

Growth takes place on potato, but is often invisible. Milk is a good medium, and in it the diplococcus produces acid and usually

causes coagulation of the casein. This coagulation is ascribed to the acid produced, but is variable, sometimes taking place within twenty-four hours, but in other cases not until after the lapse of some days. Upon blood serum the growth is very similar to that of streptococcus.

The organism is a facultative anaerobe. In artificial cultures virulence is quickly lost, and the organism often dies in from twenty-four to forty-eight hours; in dried sputum or blood, however, it has been known to survive as long as four months. It is killed in ten minutes at 52° C.

Pathogenicity.—Micrococcus lanceolatus is often present in the mouths of healthy human beings, according to Kruse and Pansini in approximately one hundred per cent. Netter found it in a condition virulent for animals in fifteen to twenty per cent. of the healthy persons whom he examined. Next to the pyogenic staphylococci and streptococci it is the most common cause of inflammations in human beings. It is probably the sole specific cause of genuine acute lobar pneumonia, and frequently induces bronchopneumonia, otitis media, meningitis, and serositis. Secondary pericarditis and endocarditis, associated with lobar pneumonia, are caused by this organism. It generally invades the body from the bronchi or lungs, sometimes from the nose, nasal sinuses, pharynx, and occasionally from the intestine (Flexner). The list of diseases which it is capable of producing is a very long one, and includes inflammations of mucous and serous membranes, abscesses in various parts of the body, mono- and polyarthritis, osteomyelitis, periostitis, parotitis, thyroiditis, nephritis, ulcerative endocarditis, and other conditions. It may cause septicæmia with single or multiple localizations. It is the most frequent cause of metapneumonic pleurisies, including empyema. Although it may be the etiological factor in the gravest diseases, it is ranked as a relatively benign organism in comparison with Streptococcus pyogenes, particularly in pleurisies and suppurations. In inflammatory exudates as well as in cultures it may die quickly or may persist for weeks and months (Welch). It is pathogenic for mice and rabbits, and to a less degree for guinea-pigs. Mice usually die in from twenty-four to forty-eight hours from a general infection, although in addition there is not infrequently an increase of organisms at the site of introduction, and special localizations may take place in the serous membranes. The blood and organs, as well as the local exudates, are filled with capsulated diplococci. Rabbits show various reactions to inoculation. According to the dose and the virulence of the organism, we may have local subcutaneous abscesses, a single or multiple serositis, or a general infection.

It is not necessary in order to obtain a general infection to introduce the organism into the blood. Sometimes large spreading phlegmons form at the site of subcutaneous inoculation, and result in perforation of the skin with evacuation of the contents of the abscess. Healing may take place or the animal may die after a long period when the organisms have almost or entirely disappeared. The micrococcus varies in its virulence not only as it is found in the mouths of healthy human beings, but also as it occurs in pathological lesions. According to Welch, the most virulent organisms are present in the freshly hepatized portion of the lung of croupous pneumonia, whereas those found in the older parts of the same process are far less active. In croupous pneumonia without secondary localizations cultures from the blood may give positive results. According to Kohn these organisms are more likely to appear in the blood in cases which result fatally than in those which recover.

Immunization.—Animals can be immunized through the use of cultures of certain grades of virulence, and also by inoculation with pneumonic sputum, and bacteria contained in inflammatory exudates, which have been exposed to temperatures approaching 60° C. to lessen their activity. Immunization has also been obtained through injections of filtrates from virulent cultures. This immunity appears, according to some authors, at the end of the third or fourth day, or, according to others, not before the fourteenth to the thirtieth day (Emmerich, Foà, Kruse, and Pansini). The duration of the immunity varies from three weeks to six months.

Mennes was able, by numerous passages from animal to animal, to obtain a culture of the pneumococcus so virulent that 0.00000001 c.c. of the blood of infected rabbits would kill other rabbits within twenty-four hours. He found that the toxicity of the culture fluid bore no relation to the virulence of the organisms. Immunity could be established by the use of either toxins or cultures, and goats, rabbits, and even horses yielded, after repeated inoculation of living cultures, a serum that possessed both protective and healing properties. Washbourn has also prepared a healing serum from the horse, but thus far it has not been tried on a sufficient number of cases of pneumonia to enable us to arrive at a decision with regard to its value in human beings. Bezançon and Griffin found that the blood serum of rabbits suffering from a local infection with pneumococcus possessed in high degree agglutinative capacity for that organism. They also found the serum of pneumonic human beings to possess similar properties.

Diplococcus intracellularis meningitidis was described by Weichselbaum in 1897 as a peculiar micrococcus, resembling the

gonococcus, which he had found in six cases of acute cerebrospinal meningitis. In morbid processes the organism appears almost always within cells. In cultures it occurs singly, in pairs, and in tetrads. Both in cultures and in tissues it is decolorized by the Gram stain. It grows on agar-agar and blood serum, and its colony and other growths resemble those of Micrococcus lanceolatus. It is only feebly resistant, and dies quickly, so that to preserve it, fresh cultures must be made at intervals of every two or three days. Similar organisms in the lesions of meningitis have been described by Jaeger, Huebner, Councilman, Mallory, and Wright, Williams, Harris, Osler, and others. It has been found in other situations, in hepatized areas of the lung, in the joints, in secretions from the nose; in myocardial abscess associated with meningitis (Boston), and in meningitis with metastatic joint lesions it has been cultivated from the blood during life (Gwyn). Councilman mentions a case of abscess of the tonsil associated with meningitis in which the characteristic diplococci were found on the cover-slip preparations and in cultures. The occurrence of the organism in the nose has led some authors to regard this avenue as the portal of entry to the central nervous system (Jaeger and Heubner). C. Fraensal has found it in these cases of pseudo-membranous conjunctivitis unassociated with meningitis.

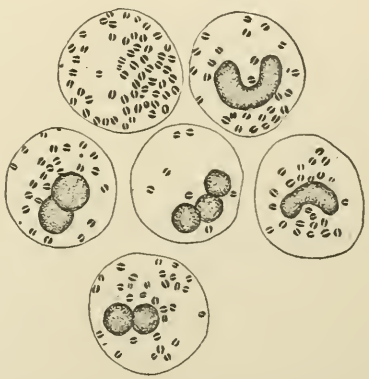

FIG. 9.—Diplococcus Intracellularis in Swollen Pus Cells from an Alveolus of the Lung in a Case of Diplococcus Pneumonia. (Councilmann, Mallory, and Wright.[19])

In cultures the organism does not give a profuse growth on any medium. According to Councilman, Loeffler's blood serum is the medium best adapted for its growth. Even when large amounts of the exudate, containing many organisms, were transplanted, single colonies only were obtained. These appear upon the serum mixture as round, white, shining, viscid-like colonies with smooth, sharply defined outlines that attain a diameter of 1 to 1.5 mm. in twenty-four hours. When the number of colonies is relatively large, they remain small, and bear a greater resemblance to the pneumococcus. The growth upon plain agar is feeble; on glycerin-agar more marked. It is often impossible to obtain a second growth upon agar-agar. The growth in bouillon is feeble, the medium becoming slightly cloudy. At the bottom of the test tube a grayish-white sediment collects, and when shaken, a viscid, string-like cloud

appears in the tube. On potato there is no visible growth, and litmus milk is said to remain unchanged (Councilman).

The organism is confined to the interior of polymorphonuclear leucocytes. It appears neither in the bodies of the tissue cells nor in their nuclei. The free cocci sometimes found in smears probably result from the rupture and disintegration of leucocytes. In localized foci of infection the meningococcus can occur associated with other organisms, these being chiefly pneumococcus, staphylococcus, streptococcus, and Friedländer's bacillus. Weichselbaum inoculated mice, guinea-pigs, rabbits, and dogs with pure cultures. Subcutaneous inoculations were without result. Inoculation in the pleural and peritoneal cavities proved fatal to guinea-pigs and rabbits. Inflammation of the serous membranes was produced. Its introduction into the meninges was followed by meningitis and encephalitis. Harris found intraperitoneal, intrapleural, and intracerebral inoculations effective in the rabbit. Councilman succeeded in producing a typical meningitis by inoculating into the spinal canal of the goat 1 c.c. of a bouillon suspension of the organism. In the exudate were pus cells containing diplococci. The majority of writers who have described this organism, since Jaeger's publication in 1895, have regarded it as the cause of epidemic cerebrospinal meningitis.

Micrococcus Gonorrhœæ, Gonococcus. *Morphology and Cultural Properties.*—The organism was discovered by Neisser in gonorrhœal pus in 1879, and was first cultivated by Bumm in 1885 on human blood serum. It is found constantly in gonorrheal pus. The cocci occur in pairs, with the adjacent sides flattened against each other, with a clear interspace (biscuit-shaped). Groups of four with the adjacent sides flattened also occur, but rarely. The average dimension of the cocci is 1 μ. In longitudinal diameter a pair varies from 0.8 to 1.6 μ, and in transverse diameter from 0.6–0.8 μ. In the gonorrheal discharge the cocci appear in small irregular groups, especially upon and within pus cells. The nucleus is not invaded, but organisms occur also free and attached to epithelial cells. The greatest number of organisms is found not in the earliest serous secretion, but in the later purulent discharge. By Gram's and Weigert's stains the organism is decolorized, and by this characteristic is distinguished from other pyogenic cocci, excepting the meningococcus. It is facultative anaerobic, and grows only at the temperatures approaching that of the body. Growth cannot be obtained on nutrient gelatin and only with difficulty and very rarely upon plain agar. Coagulated human blood serum affords the best basis for media. The mixture of Wertheim, which consists of human blood serum and nutrient agar in the proportion of one of the former to two or three of the latter, has afforded

excellent results. The addition of sterile human urine improves the serum-agar mixture (Steinschneider). Flexner has found that a medium made from macerated pig's fœtuses and mixed with agar is suitable for its growth. The colonies are usually discrete. When upon the surface of the medium they are pale, grayish, translucent, finely granular, with finely notched borders. In a mixture of bouillon and blood serum a membrane is formed upon the surface, leaving the fluid clear. The developed colonies scarcely attain to 1 mm. in diameter. The organism, as a rule, dies out very quickly, and it is difficult to obtain successive generations of cultures. Cultures on serum agar, however, which were prevented from drying, have been known to survive as long as forty-five days. Weak disinfectants, and temperatures above 42° C., as well as drying, quickly kill the organism.

Fig. 10.—Gonococci. *a*, From a pure culture, about × 1,000; *b*, gonococci in pus cells and an epithelial cell; *c*, schematic representation of the form and mode of division of gonococci. (Bumm.)

Pathogenicity.— The gonococcus is a strictly human parasite. It is found in the discharge in cases of gonorrhœa, and in the several mucous membranes where this condition is present. It has been demonstrated quite frequently in films and obtained in cultures from the urethra, urinary bladder, uterus, Fallopian tubes, conjunctiva, and joints, and occasionally from periurethral, ovarian, and subcutaneous abscesses. Cushing has isolated it in two instances of diffuse peritonitis, originating in a gonococcal salpingitis, and Leyden and Michaelis more recently in another instance of a peritonitis. It has been cultivated from the blood during life by Blumer, Thayer, and Lazear, and demonstrated at autopsy in the same cases in the lesions of ulcerative endocarditis, etc. (Flexner). The endocarditides associated with gonorrhœa are commonly caused by the gonococcus, and in these cases a general infection with the organism may take place. Pericarditis, pleuritis, and suppurative myocarditis also may be caused by the gonococcus. Ahlfeld has described an instance of gonorrheal infection of the mouth in an infant at birth. The affection ran a harmless course, but in the intensely yellow deposit upon the hard palate typical gonococci were found. Epididymitis (Collan) and acute hydrocele (Gross) have been shown to be caused by the invasion of gonococci. Hansteen demonstrated gonococci in a spreading periglandular phlegmon of the inguinal glands associated with gonorrhœa.

The gonococcus is in general non-pathogenic for animals, but when

inoculated with bits of agar into the eye or into the peritoneal cavity of mice or guinea-pigs it may cause suppurative inflammation. Wassermann has succeeded in killing mice by injections of dead cultures into the peritoneal cavity. He believes that the gonococcus produces a poison that is analogous to the cholera poison described by Pfeiffer, in that it is intimately associated with the bodies of the bacteria. In minute quantities it produces local inflammation, fever, swelling of the adjacent lymph glands, and pains in the muscles and joints. Immunization against this poison has not thus far been successful. According to Christmas, the toxin resists heating to temperatures of from 50° to 70° C. and is precipitated by alcohol, the residual fluid becoming freed from toxicity. Cultures extracted by means of glycerin at a temperature of 50° C. yield a toxic extract which is pathogenic for animals.

A *pseudo-gonococcus* was first obtained by Lustgarten and Mannaberg from normal male urethræ. Gram's stain was not employed, and as among their cultures Staphylococcus aureus was noted, very little significance attaches to their observations. Hogge and Noguès and Wassermann have described a coccus which agrees with the gonococcus in form and staining-reactions, but differing in its cultural properties. Hogge found the organism in the urethra of a man who had never had gonorrhœa, and Noguès and Wassermann obtained it in a case of inflammation of the prostate and of the adjacent portion of the urethra. The coccus grows readily and abundantly upon all culture media except potato; it does not liquefy gelatin. It is non-pathogenic for guinea-pigs. Prussenberg has described a pseudo-gonococcus, which grows upon Loeffler's blood serum and in bouillon containing blood, not, however, upon agar. It decolorizes by Gram, and is pathogenic when injected intraperitoneally into guinea-pigs.

Micrococcus Tetragenus. *Morphology and Cultural Properties.*—This organism was isolated by Koch and Gaffky in 1881 from phthisical cavities. Its characteristic growth is in the form of tetrads enclosed in gelatinous capsules. It stains by Gram's method, and grows on all culture media at the room temperature, with or without oxygen. It forms elevated, white, non-liquefying colonies on gelatin. Under the low powers of the microscope these appear as circular or lens-shaped grayish-yellow discs, the surface of which is granular and mulberry-like, the edge appearing uniform or slightly indented. From the surface of the medium the colonies project somewhat, and reach an average diameter of from 1 to 2 mm. In stab cultures the colonies become confluent, forming a thick, white, adherent growth, upon the surface of which is a membrane 4 to 5 mm. in diameter. On the surface of agar and coagulated blood serum the growth is

gelatinous, abundant, grayish-yellow in color, and strongly resembles the growths of the capsulated bacilli. Growth takes place upon potato as a thick gelatinous carpet. The individual organisms average about 1 μ in diameter. Bouillon cultures become rapidly clouded and a precipitate forms. In artificial cultures, besides the tetrads which are most common, one sometimes finds large spherical individual cocci.

Pathogenicity.—The organism is often found in phthisical cavities, being often situated in their walls. Deléarde found it in almost pure

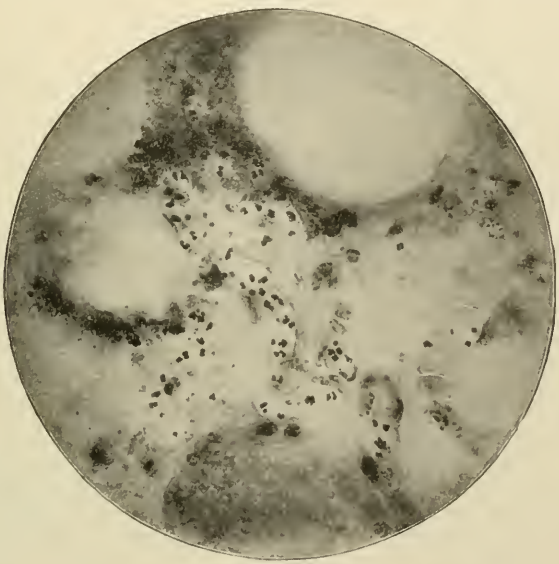

FIG. 11.—Micrococcus Tetragenus, in a Section of the Lung of a Guinea-pig. × 500. (Fraenkel and Pfeiffer.)

culture in the sputum of bronchopneumonic patients. Bosc and Galaville obtained it in almost pure culture from a case of gangrenous pneumonia; not uncommonly it produces abscesses in human beings, having been found associated with carious teeth, and in suppurations of the nose, mouth, and neck. Its relation to suppurative processes in this region is accounted for by the fact that it is commonly found in the saliva and in the nasal secretions even of healthy individuals. It has been isolated, moreover, from the contents of serofibrinous and purulent pleuritides, from pericardial exudates, and circumscribed suppurative meningitides (Netter, Benoit, Bezançon, etc.). Chauffard, Ramond, and Netter have described several instances of fatal septicæmia due to this organism, and Lartigau has attributed rare cases of angina to it.

Small quantities of cultures introduced subcutaneously into white mice cause a uniformly fatal disease. Death occurs at the end of the third to the sixth day, and the organisms are found in the blood-vessels, chiefly of the spleen, lungs, kidney, liver, and less abundantly in the blood of the heart. Gray mice are far more resistant. Guinea-pigs and rabbits succumb to intraperitoneal inoculations (when purulent or fibrinous inflammations develop), and to intravenous injections. Intratracheal injections in guinea-pigs set up fatal pneumonia and bronchitis. Cold-blooded animals and dogs are immune. Boutron proposes the name "Micrococcus tetragenus septicus" for the Koch-Gaffky micrococcus to distinguish it from other similar and non-pathogenic tetragenous cocci.

Micrococcus melitensis was discovered by Bruce, Hughes, and Westcott in 1883, and obtained in cultures from the blood and the organs of persons dead of Malta fever. The growth upon agar-agar is very slow. It has also been obtained *intra vitam* from the spleen. The organism is a very small coccus, occurring singly and in pairs, and almost never in short chains. It is decolorized by Gram's method, and grows best upon neutral agar-agar. Its growth upon gelatin is very slight and this medium is not liquefied. The micrococcus is non-pathogenic for mice, guinea-pigs, and rabbits. In apes it gives rise to fever, which may last three weeks before death occurs; after death it can be cultivated from the organs. Kretz and Musser and Sailer have utilized the agglutination reaction of blood serum with the cultures of this coccus for the recognition of Malta fever. Kretz found the blood serum in the case which he examined to act rapidly in dilutions of 1:300 and slowly in dilutions of 1:1,000.

Bacillaceæ.

Vegetable microorganisms, approximately rod-shaped, in which the longitudinal diameter is in excess of the transverse diameter. Generally simple, unbranched rods. Usually straight and of a uniform diameter, but sometimes slightly bent or curved, or irregularly swollen. They stain uniformly or irregularly with the deeply tinged points distributed regularly or irregularly in the course of the rod, or accumulated at the poles or in the more central portions. Filamentous forms are rare. The organisms occur singly, in pairs or chains, or united to form spiral or conglomerate masses. Both motile and non-motile forms are known, the latter for the most part being provided with flagella. The pathogenic varieties are associated with specific diseases, and also with non-specific inflammatory, necrotizing, and other pathological conditions.

Group of B. Coli Communis and B. Typhosus.

Medium-sized, motile bacilli which show little tendency to the formation of threads. Readily stained with anilin dyes, and as readily decolorized by Gram's or Weigert's methods. Non-sporogenous. With very few exceptions, they are facultative anaerobic organisms. They grow upon all culture media readily, but are incapable of liquefying gelatin. Motility by means of flagella. Pathogenicity is present in most members, and well marked in some. They are extensively disseminated in nature, some being constant inhabitants of the animal body (B. coli communis), while others occur in association with widely distributed infectious diseases (B. typhosus).

FIG. 12.—Bacillus Coli Communis; a Pure Culture from an Agar Plate. × 500. (Lehmann and Neumann.)

BACILLUS COLI COMMUNIS.—First isolated by Emmerich from the intestines and internal organs of patients suffering from cholera asiatica, and regarded by him as the specific cause of the disease. It was also described by Buchner, and afterwards (1886) isolated from the fæces of infants by Escherich. Weisser identified the organism as a common intestinal bacillus. B. coli communis is probably identical with the microorganism obtained by Passet from an abscess, which he had called B. pyogenes fœtidus.

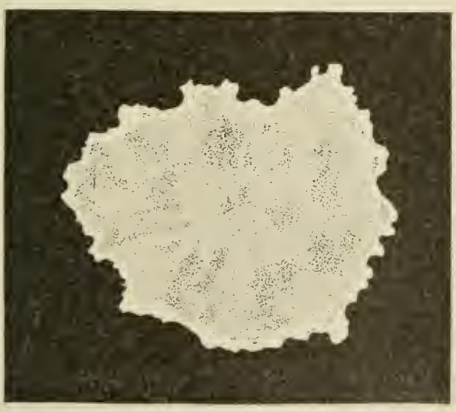

FIG. 13.—Bacillus Coli Communis; a Superficial Colony on Gelatin Plate. Four days at 22°. × 60. (Lehmann and Neumann.)

Morphology and Cultural Properties.—The bacilli average from 0.4 to 0.7 μ in width, and from 1 to 3 μ in length. For the most part they appear in the form of short rods with rounded ends, but coccus-like examples on the one hand and threads 6 μ in length on the other are sometimes found. The bacilli not infrequently appear in pairs (diplo-bacilli), and very rarely in pseudo-chains consisting of several individuals. Irregular degeneration forms presenting vacuoles, polar staining, and irregularly notched sides are sometimes found in pathological lesions and in old cultures. Spore formation is unknown.

Cultures are killed in from ten to thirty minutes at a temperature of 60° C. The organisms do not resist drying for any length of time, and are readily destroyed by chemical disinfectants. Motility is present in fresh cultures, but often is demonstrable only in the first few hours of growth in the thermostat. Only rarely do cultures twenty-four to forty-eight hours old still exhibit motility. The motion is slow, and usually not all the individuals in the field move at the same time. Flagella are present, their number varying (according to different authors) from four to many. In rare instances a single polar flagellum has been observed. These are demonstrable by Loeffler's and Van Ermengem's methods, and in some preparations they appear to arise from a capsular investment. Both Gram's and Weigert's stains give negative results. The organism grows at low as well as high temperatures on all media. It is a facultative anaerobic.

Gelatin.—The colonies in the depth are small, round, yellowish or brownish in color, and quite homogeneous. As they grow older they enlarge and become more opaque. The surfaces of the colonies upon gelatin are spreading, the central nucleus is relatively dark, and round or lens shaped, while the peripheral portion of the colony shows a bluish translucence, the edges being irregularly crenated; a concentric structure is present in some colonies. In the early stages they present a mother-of-pearl-like reflection, while later on they become thicker and cartilage-like. In the stab culture the most common form is that of a nail with a flat head. The superficial extension often reaches the sides of the tube, and growth takes place throughout the entire length of the stab. The superficial layers of the gelatin are sometimes rendered cloudy, and gas formation appears if sugar in demonstrable quantities is present in the medium. The growth is less abundant and opaque than is observed with B. lactis aerogenes, and the medium does not turn brown, as in the case of the pneumobacillus (see below). No liquefaction takes place. Upon soft media irregular projections appear in the colonies bringing about resemblance to B. proteus.

Agar-agar.—The colonies upon agar-agar in general resemble those upon gelatin. They are usually, however, of more rapid development on account of the higher temperature at which this medium can be kept, are more opaque, and do not tend to spread on the surface of the medium to quite the same extent as in gelatin. The individual colonies are, therefore, somewhat thicker and less translucent. The agar stab also exhibits a nail-like growth. Crystals are common in old cultures.

Bouillon.—Bouillon is rendered cloudy. The sediment, which

varies in amount, forms after one to two days, while upon the surface of the medium a membrane may appear. The reaction of the medium is determined by the presence or absence of sugar; it becomes acid when sugar is present, and alkaline when it is absent (Wurtz). A slight and somewhat offensive odor is developed in the bouillon. Indol is formed, but no phenol. Reduction phenomena, as exhibited in the conversion of nitrates into nitrites and the decolorization of litmus and indigosulphate of sodium, are observed.

Potato.—The growth is usually abundant, and of a brownish, yellowish, or white color. Sometimes, however, it is so slight as to be scarcely visible. The surrounding medium is generally discolored by a brownish-yellow pigment produced in small quantity.

Litmus-milk is first rendered red (acid production), and afterwards is coagulated. The coagulation may take place at the end of twenty-four hours, or may be delayed for several days or even weeks. The acids formed are chiefly acetic, lactic, and formic.

Fermentation.—Various sugars—glucose, dextrose, maltose, and saccharose—are fermented. Some varieties, however, while fermenting the other sugars, are incapable of acting upon saccharose (see Bacillus icterogenes, page 649). Opinions are still divided as to its capacity to cause hydration of starch. Glycerin also is fermented. For the demonstration of the ferment capacity of the organism stab cultures in glucose-agar are well adapted. There appears at the end of twenty-four hours, at the temperature of the thermostat, a rich development of gas, which has fractured the medium, and perhaps driven portions of it to the top of the tube. The gas formed consists of carbon dioxide and hydrogen—usually in quite definite proportions, as pointed out by Theobald Smith.[21] Growth takes place in albumin-free media (Uschinsky's fluid).

Pathogenicity.—B. coli communis is widely distributed in nature. It is a constant inhabitant of the intestine of human beings, as well as of many of the lower animals. It is a frequent invader of the internal organs in all kinds of diseases, especially when intestinal lesions are present. In many of these cases, however, it manifests no definite pathogenic action, and is without clinical significance. On the other hand, however, there is no doubt that it is often pathogenic for man, and plays an important part in inflammations of the urinary tract and biliary passages, and also, but usually with less independence, in peritonitis and appendicitis. It has been looked upon as an active pathological factor in diffuse disease of the intestine. Epidemics of infectious enteritis have been referred to a modified and more pathogenic form of this organism by Gilbert and Girode and Rossi-Doria, and sporadic cases of so-called cholera nostras have been attributed to

it by Hueppe. Furthermore, Booker, Baginsky, and Escherich have regarded it as concerned in the production of cholera infantum, and some authors believe that it has to do with the causation of dysentery (Escherich). In diffuse as well as circumscribed peritonitis it has been found alone, but in the great majority of these cases it is associated with other organisms, chiefly the pyogenic micrococci. The experiments of Barbacci on dogs would indicate that in perforative peritonitis the primary lesions are caused by pus-producing cocci, while the growth of B. coli communis, taking place later, is frequently so exuberant as to obscure the pyogenic organisms. Infection of the biliary passages is caused by B. coli, and the organism may be found in the interior of gall-stones, with the formation of which it may be concerned (Welch). It has also been repeatedly found in abscesses of the liver, in cystitis, and pyelonephritis, and, in fact, is the bacterium most commonly associated with the last two conditions. The following pathological conditions have also been attributed to it: puerperal infections, endocarditis, meningitis, tropical abscess of the liver, bronchopneumonia, putrid bronchitis, chronic amygdalitis, strumitis, empyema of the tear-ducts, cutaneous and subcutaneous suppurations, paronychia, emphysematous phlegmon, and surgical wounds. In all these classes of cases the colon bacillus has been found either alone or in association with other pathogenic microorganisms. In the internal organs it often appears in clumps suggesting zooglœa.

But although we cannot deny that this organism may be capable by itself of pathogenic action in man, it would seem certain that this is for the most part of a mild character, and that a more important part played by the organisms consists in invading territory already damaged or previously occupied by other bacteria. Not a few instances are recorded in which this bacillus had been present throughout the body and has produced a general infection, starting probably from local lesions upon the surface of the body (Sevestre), from an angiocolitis (Netter, Flexner), or from a cystitis (Sitmann and Barnow). Experimental animals are susceptible to inoculation. Great differences are exhibited according to the amount and source of the culture injected. Relatively large quantities of cultures of the bacillus obtained from indifferent sources are required to kill even small laboratory animals, so that it is probable that death in these cases is caused by intoxication rather than infection. Mice, for example, may resist injections of 1 c.c. of bouillon cultures into the peritoneal cavity, while 0.1 c.c. of the culture of the organism obtained from some pathological lesion may cause death in from twenty-four to forty-eight hours. Rabbits succumb to intraperitoneal and intravenous inoculations, but large quantities of the organisms are usually required. If

the animal dies soon after inoculation, the organisms are still found at the site of injection as well as in the organs; but death may be delayed until all of the organisms have disappeared, and is then to be attributed to their toxic products. Enteritis of a serous type, with perhaps swelling of Peyer's patches, is a common result of intravenous and intraperitoneal inoculations, and the latter often produce a fibrinopurulent peritonitis. The least effectual mode of inoculation is by subcutaneous injections. Abscesses, however, may be developed at the site of the puncture, but unless considerable quantities of the organisms have been injected, only very few are found in the internal organs. Dogs and often rabbits resist relatively large injections into the peritoneal cavity, unless the amount of fluid introduced is greater than can be absorbed in a given time, or unless some foreign body, as for example potato or sterilized fæces, has been introduced at the same time when peritonitis develops. Ackermann has produced osteomyelitis in young rabbits by intravenous injections of B. coli. Welch and Blackstein, as well as Gilbert and Lion, observed after intravenous inoculations the production of multiple foci of necrosis in the liver of the rabbit. Cultivation of the organism upon artificial media deprives it of its virulence, which can, however, be restored by successive passages through the animal body.

Immunization.—Animals can be immunized to B. coli communis. Their blood serum is then protective to other animals, and even in considerable dilutions possesses the property of agglutinating suspensions of the organism. Ordinary human blood serum also causes agglutination of B. coli communis, as has been shown by Courmont, Widal, and Nobécourt. This property, however, of causing agglutination, which is present in normal human serum, varies according to the origin of the serum and the variety of the bacilli. This fact, taken in conjunction with the observations on the variations in the biological properties of the organism, affords an additional reason for regarding the characters ascribed to it as representing those of a group rather than of a particular microorganism. Widal considers that the application of the serum diagnosis may provide a clinical basis for distinguishing the chief groups of B. coli. Lesage has observed this reaction for a particular group of the bacillus which occurs in infantile diarrhœas.

Bacillus Typhosus. *Synonyms.* — Typhoid bacillus, Eberth-Gaffky bacillus.

This organism was first observed in sections of the organs of persons dead of typhoid fever by Eberth and Koch. It was cultivated by Gaffky (1884). Although complete proof establishing this bacillus as the cause of typhoid fever has not thus far been adduced, yet

its constant occurrence in association with the lesions of that disease, and its absence from pathological conditions unassociated with typhoid fever, leave little doubt of its causative rôle. The lack of complete proof is due to the fact that in the lower animals conditions resembling typhoid fever do not occur.

Morphology and Cultural Properties.—The bacilli are actively motile. They vary from 0.5 to 0.8 μ in diameter, and from 1 to 3 μ in length. They occur singly, but also show tendencies in cultures to grow into pseudo-threads. In the organs of human beings they occur for the most part in zoogloea-like masses. Their size varies somewhat, according as growth has taken place in the animal body or upon certain culture media. They are apt to be somewhat smaller in size when grown upon gelatin than upon potatoes, while at low temperatures the tendency for thread formation is particularly marked. Some difficulty is met with in staining the bacillus, both in films and in tissues, although the ordinary anilin dyes usually suffice, provided only that care is exercised that decolorization be not too actively carried out. Gram's and Weigert's stains are negative. Cultures grown upon potato examined in the fresh state show highly refractive polar bodies, which take a more intense stain; in other cases polar and central vacuoles are observed. The latter by some authorities have been regarded as spores; but inasmuch as organisms

FIG. 14.—Bacillus Typhosus, from a Gelatin Colony. Impression preparation. × 1,000. (Fraenkel and Pfeiffer.)

in which they are present show no greater resistance than those in which they are lacking, and since these appearances are especially numerous in cultures grown under unfavorable conditions, they are probably to be regarded as consequences of involution. The form of motility depends upon the character of the growth. The single small individuals show a lively pendulous motion, whereas larger thread-

like forms have a serpentine and deliberate movement. Motility depends upon the possession of a variable number (ten to eighteen) of flagella that are arranged around the periphery of the organism (peritricha). The flagella are stainable by Loeffler's method, and also by that of Van Ermengen. The bacillus is readily killed at temperatures of 60° C. in from one-half to one hour. Drying in thin films, according to Kruse and Paffenholz, is destructive in from five to

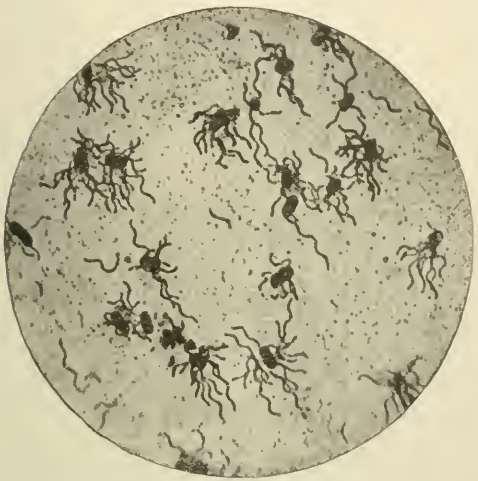

Fig. 15.—Bacillus Typhosus, Showing Flagella. × 1,000. (Fraenkel and Pfeiffer.)

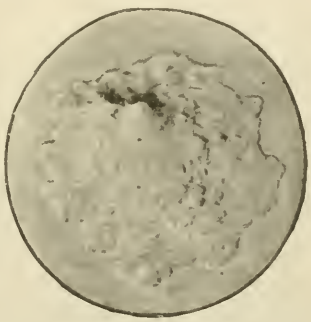

Fig. 16.—Bacillus Typhosus—Colony in Nutrient Gelatin. (Roux.)

fifteen days, but Gaffky and Pfuhl state that the bacilli were not dead at the end of three months, and Billings and Peckham found them alive on threads at the end of seven months. Frozen in ice the bacillus retains its vitality (Prudden).

The typhoid bacillus grows upon all ordinary culture media, and best at the body temperature. Between 9° and 15° C. growth is slight. At 42° C. it is also diminished. Growth takes place best in the presence of oxygen, although the organism is a facultative anaerobe. In general the condition of growth upon culture media is similar to that of B. coli communis, the chief difference being that it is somewhat slower and less abundant than in the latter.

Gelatin.—Deep colonies on gelatin plates are round or lens shaped with sharp edges, almost homogeneous, and of a yellow or brownish color. The young superficial colonies are translucent, iridescent, with irregular, grape-leaf-like indentations. Slight magnifications show them to be almost homogeneous, or marked by delicate elevations and depressions. As the colonies grow older the centre becomes darker, and the depressions tend to disappear. In stab cultures a nail-like growth is obtained, although the membrane on the surface of the medium tends gradually to reach the edge of the glass.

Crystals sometimes appear in old cultures. No liquefaction occurs. Upon soft media a marked tendency, greater than with B. coli, to send out lateral and branching processes is observed.

Agar-agar.—Individual colonies are somewhat coarser than in plates upon gelatin; the surface growth is more opaque, and the characteristic markings of the colony are less evident. Stab and slant cultures grow somewhat more abundantly than upon gelatin, because of the higher temperature at which this medium can be incubated, and the resulting growth is more opaque. Even upon slants growth extends only for a millimetre or two beyond the line of inoculation.

Bouillon.—This medium becomes uniformly cloudy, perhaps somewhat less intensely so than the colon bacillus, and a membrane is not formed. Indol is usually absent from cultures in bouillon and in peptone solution, although in suitable (peptone) media, produced by tryptic digestion, indol may also be developed by this organism, as has been pointed out by Peckham. This indol-producing capacity is not developed at once, but is gradually acquired.

Potato.—This medium produces variable results. The most typical is that originally described by Gaffky as an invisible growth which covers the entire surface of the potato, and which, upon being touched with a platinum needle shows a certain degree of resistance. In some cases, however, the growth remains localized to the region of the inoculation, and assumes about the same color as that of the potato, while at other times it may be rich, covering the entire surface of the potato, and elevated for a millimetre or more, while assuming a yellowish or brownish coloration. Occasionally the potato is discolored and takes on a greenish tint. The variation depends upon the composition of the potato, and is more or less influenced by the degree of acidity or alkalinity, although it does not depend entirely upon this property. Where the growth is abundant and colored, there is but little difference between it and the growth of B. coli communis.

Litmus-milk.—This medium serves for the differentiation of B. typhosus from B. coli communis. The typhoid bacillus grows abundantly in it, fails to produce coagulation even at the end of many weeks, and forms so little acid as to produce only a faint lilac tint. After this preliminary acid production, some forms produce alkali to such an extent as to bring about a deep blue coloration of the medium. As the typhoid bacillus is incapable of splitting up lactose it is probable that the small amount of acid originally produced comes from traces of dextrose contained in the medium.

Blood Serum.—Upon this medium B. typhosus shows a ready growth, similar to that of B. coli communis. No liquefaction occurs.

Fermentation.—None of the sugars—glucose, saccharose, lactose, —is fermented, neither is glycerin split up. Glucose is converted into acid, but there is no gas production.

Reduction is effected, although less actively than by B. coli communis. Litmus is decolorized, and indigosulphate of soda and nitrates are also reduced, but in very small amounts.

Pathogenicity.—In human beings B. typhosus is the stated cause of a specific disease—namely, typhoid fever. Its special foci of localization and development are the nodular lymphatic structures of the intestines and of the mesentery. Very soon the organism gains entrance to the spleen, the bone marrow, liver, and gall bladder, where a rich development also takes place. In the spleen and abdominal lymphatic glands the bacilli exist in zooglœa-like masses. More rarely the lungs, kidneys, and central nervous system become the seats of development. One of the especial properties of the bacillus is to cause proliferation of lymphatic and endothelial cells, a peculiarity upon which some of the characteristic lesions of typhoid fever depend. The hyperplastic organs in certain stages of the process undergo necrosis, which in the intestines is followed by the characteristic lesions of the disease—the typhoid ulcers. A similar necrosis of tissue, either microscopic or macroscopic, appears in the abdominal glands, spleen, and liver. In the last organ these foci are of microscopic size, very numerous, and are generally known as "lymphoid nodules." The bile is a favorite breeding-place for the organisms, which, having once gained entrance, may persist for months, or even years, after the original disease has disappeared (Welch, Flexner, Cushing, Miller, and others). One of their accessory effects is the production of gall stones, which have been also experimentally produced in rabbits (Gilbert, Richardson). From the kidneys the bacilli may be excreted with the urine. That they show a tendency to persist for a long time in the urinary bladder is proved by the fact that they may continue to be voided for weeks after convalescence from typhoid fever is established, even in cases in which the urine is perfectly clear and free from pus (Richardson). In purulent urine the bacilli have persisted for two years (H. K. Young). Special localizations of the organism may take place, as in the peritoneum, meninges, and bone, producing acute inflammations. A common post-typhoid osseous infection is periostitis with cortical ostitis, in which case the ribs are more often affected than other parts of the osseous system. In rare cases abscesses in voluntary-muscle tissues around joints, in the thyroid gland, the epididymis, and kidney have been ascribed to this bacillus, and it has also been found in inflammations of the joints, the parotid gland, and the middle ear, and in ovarian

cysts. Dmochowski and Janowksi have demonstrated experimentally the pyogenic action of B. typhosus.

In the last few years we have come to know cases of typhoid fever without enteric lesions. On clinical grounds this point had already been mooted by Sidney Phillips and J. W. Moore. Recently Chiari and Kraus, Flexner, Hodenpyl, Nicholls, and Keenan have examined into this question. Besides the ordinary forms of typhoid fever, a type which is termed typhoid septicæmia, in which no special local manifestations of bacilli are found, may be distinguished. Another form includes those instances of localizations other than enteric. The organs attacked may be the spleen, the kidneys, or the cerebro-spinal meninges. These cases are designated as examples of pneumo-typhoid, nephro-typhoid, cerebro-spinal typhoid, and spleno-typhoid. Typhoid bacilli do not generally appear in large numbers in the circulating blood, and it is the exception to find them at all in blood cultures made during life with proper precautions, although not a few such instances are on record. In the majority of the positive cases considerable quantities (one or more cubic centimetres) of the blood were used for making the cultures. The bacilli may be transmitted directly from the mother to the fœtus, in which case the fœtus may be still-born, or live for some time after birth. Ernst described an instance in which the child four days after birth, by icterus and an exanthem, showed evidences of such a general infection. Localized lesions due to the presence of these organisms in the fœtus have thus far not been demonstrated. The typhoid bacillus does not possess specific pathogenicity for experimental animals. Mice, guinea-pigs, and rabbits succumb to inoculations of this organism very much as with those of B. coli communis. Subcutaneous inoculations into rabbits and dogs have produced localized abscesses. When death has occurred in inoculated animals the clinical and pathological pictures point to an intoxication rather than to an infection, and similar fatal intoxications can be produced by cultures sterilized by heating or filtration (Sirotinin). As is the case with B. coli communis, although these animals exhibit intestinal lesions, the special lymphatic apparatus of the intestines and of the abdominal cavity do not show any particular involvement.

Poison Production.—Brieger and Fraenkel have separated from bouillon cultures a poison belonging to the group of toxalbumins which they call typhotoxin. The chief poison, according to Pfeiffer, produced by the typhoid germ is intimately bound up with the proteid of the bacterial cell, and goes over in small quantities only into the fluids in which the bacilli are cultivated.

Immunization.—Mice, guinea-pigs, rabbits, dogs, and goats can be

immunized by injections of gradually increasing amounts of living or sterilized cultures. The blood serum of such immunized animals possesses protective and healing properties, which, as many observations go to show, are also found in the blood serum of human beings who have recovered from typhoid fever. According to Pfeiffer this protective property is specific, is produced by the typhoid bacillus, and is active only against processes set up by this organism. Allied conditions due to the colon bacillus group are not influenced. The attempt has been made to use such protective and healing sera in the treatment of typhoid fever in human beings, but thus far their value has not been settled. Wright and Semple have immunized human beings to the typhoid bacillus by the subcutaneous injection of sterilized cultures. The blood serum of these individuals possessed immunizing and agglutinating properties. The capacity to bring about agglutination is commonly observed in the blood serum of human beings during an attack of typhoid fever, and during and after convalescence; this also appears in the case of animals which have been immunized. Van de Velde injected a horse, at intervals for two years, with typhoid bacilli. At the end of twelve months the agglutination capacity of the serum was 1:1,000,000, whereas the serum of healthy animals untreated showed no action in dilutions of 1:20.

Demonstration of Typhoid Bacillus in Dejections of Human Beings.—The dejections of human beings suffering from typhoid fever contain typhoid bacilli. The isolation of these bacilli, owing to the admixture of B. coli communis, B. proteus, etc., is rendered difficult. Special media, among the best of which are those suggested by Elsner and Capaldi, should be employed (pages 580 and 585). The more characteristic colonies developing upon these media are transferred to glucose-agar and incubated for twenty-four hours. Only such specimens as do not form gas are tested further. Elsner succeeded in isolating the bacilli from stools in fifteen out of seventeen cases, at stages of the disease varying from the seventh day to the sixth week. Lazarus obtained positive results as late as the eighth and ninth weeks. Many other bacteriologists report favorably upon this method, although instances of failure have not been uncommon.

Bacillus Dysenteriæ (Shiga).—Found by Shiga in an epidemic of dysentery in Japan, and believed by him to be the cause of this disease. A similar bacillus has been found by Flexner and Barker in the dejecta, and at autopsy in the intestines, in numerous cases of acute tropical dysentery occurring among the American soldiers in Manila.

Morphology and Cultural Properties.—The bacillus is a short rod

with rounded ends, asporogenous, occurring singly and in pairs, very rarely as more elongated threads. It shows great similarities with the typhoid bacillus. The organism stains in the usual dyes, and with methylene blue shows deeply staining polar bodies. The Gram and Weigert stains give negative results. The organisms are moderately motile. In old cultures are seen pronounced involution forms, consisting of small coccus-like fragments, larger irregularly swollen rods which are often joined together in short chains, and bent or twisted threads with sausage-like swellings. Growth takes place at the room temperature, but somewhat better in the thermostat. On gelatin plates at room temperature the individual colonies appear, after two or three days, as round points which under a low magnifying power appear faintly yellow and finely granulated. At the end of five or six days they reach their full size and the deeper colonies then show a central nucleus and a lighter granular periphery, whereas on the surface the spreading is more pronounced at the peripheral portions of the colony. In stab cultures the growth takes place along the entire track of the needle. Gelatin is not liquefied. Colonies in agar-agar at the end of twenty-four hours in the incubator are larger than when gelatin plates are employed, those in the depth appearing round or lens-shaped, white, semi-opaque, and faintly granular, whereas those on the surface show a central nucleus with a slightly spreading margin, white in color and of a soft consistence. The fully developed colonies may reach a size of two to three millimetres in diameter. The growth upon glycerin-agar is somewhat less abundant than upon ordinary agar. Growth upon blood serum is abundant, but not particularly characteristic. Gas is not formed in any of the sugars. Bouillon is rendered diffusely cloudy, and after some days a slight sediment appears at the bottom of the tube. A membrane does not form upon the surface, although in old cultures fragments of growth may reach the surface, and are likely to adhere at the edges where the fluid and the glass meet, especially in the Erlenmeyer flask. On potato the growth varies, being sometimes invisible or nearly so, although cover slips will show a growth spreading over the entire surface. At other times, a yellowish-brown membrane appears, and by the end of a week may have increased considerably in thickness. The potato is not discolored. Indol is usually not demonstrable until the end of several days; some varieties of bacilli seem to make it even more slowly or not at all. Litmus milk is slightly acidified, but coagulation does not occur even after several weeks.

Pathogenicity.—Shiga observed the organism in thirty-four cases in the dejecta of persons suffering from dysentery, and in two fatal

cases in the intestinal wall and in the mesenteric glands. It was never found in the dejections of healthy individuals, nor was it discovered in any one of several cases of severe diarrhœas in the tropics examined by the writer, who confirmed Shiga's findings in dysentery. That the organism is pathogenic is further indicated by the fact that it is agglutinated by the diluted blood serum of persons suffering from dysentery. Small animals, such as the mouse, guinea-pig, rabbit, and cat, succumb to subcutaneous and intraperitoneal inoculations. Guinea-pigs, to whom after previous alkalinization of the gastric juice bouillon suspensions of the organism have been administered through a catheter, succumb after about five days. At autopsy the small intestines are found to be hyperæmic and to contain bloody and mucous material from which the bacillus can be recovered in pure culture. Cats to whom croton oil has first been administered and later cultures of this bacillus develop a diarrhœa, the dejecta containing the organism. In one instance in which death had occurred at the end of four weeks, the rectum showed an excessive mucous discharge from which the bacilli could still be cultivated (Shiga). The introduction of the organism into the stomach of the dog was followed by diarrhœic stools containing the bacilli, and death on the fifth day, the upper portion of the small intestine being found injected and filled with mucous contents. Hemorrhages had taken place into the intestinal wall; the mucous membrane of the large intestine was softened and covered with mucus. B. dysenteriæ was recovered from the mucus in the small intestine. The dead cultures are pathogenic for guinea-pigs, rabbits, and mice.

Immunization.—Small animals can be immunized by the injection of cultures killed by being kept for twenty minutes at a temperature of 60° C. The blood serum of these animals develops agglutinating properties. Shiga injected cultures of the dead organism subcutaneously into himself, and found that his blood developed agglutinating properties. He has recently obtained a serum from larger animals which he believes proved of distinct benefit in the treatment of a small number of cases of human dysentery.

Bacillus Paradoxus.—Obtained by Kruse and Pasquale from a case of gangrenous dysentery which they examined in Alexandria and from the liver of which they obtained this organism in pure culture. It is possible that this organism may be identical with B. dysenteriæ. It resembles the typhoid bacillus in its general properties. The parallel cultures upon potato showed slight differences. The chief differential point is in connection with the indol reaction, which is positive with this organism.

Bacillus Meningitidis.—This was first obtained by Neumann and

Schaeffer from a case of meningitis. It is closely related to the typhoid bacillus from which it differs chiefly in its behavior in cultures on potato. It does not ferment any of the sugars. In animals it exhibits pyogenic properties probably more marked than those of B. typhosus. As there were no typical typhoid lesions in their cases these authors considered the organism as probably distinct from B. typhosus—a conclusion that is not borne out by our present knowledge.

Bacillus Pseudo-Typhosus (Kruse).—Under this heading Kruse groups bacteria closely resembling B. typhosus, which have, however, been found under conditions in which B. typhosus was supposed to be absent. Careful analysis of the cases suggests a different conclusion; and, at all events, the designation is badly chosen.

A pseudo-typhoid bacillus was described by Pansini, who found it in four cases of abscess of the liver, three of which followed dysentery. No essential difference (serum reaction not tried) could be found between this organism and B. typhosus. Babes obtained on one occasion a similar organism from a case of dysentery. It is highly probable that these organisms are identical with or closely related to B. dysenteriæ.

Lösener isolated an organism similar to the foregoing from the cadaver of a pig that had been infected with tetragenus. He obtained a second from the spleen of a case of typhoid that had lain for ninety days in the peritoneal cavity of a pig's cadaver, and a third out of a sample of soil taken in the neighborhood of Berlin, a fourth from the Spree water supplied to Berlin, and a fifth from an outhouse which, so far as was known, had been used only by healthy human beings. Babes obtained a second specimen from the cadaver of a mouse, and a third from non-suspected water.

The observation of Remlingler and Schneider on the occurrence of typhoid bacilli in external nature (in water and earth), and also in the intestinal canal of persons free from typhoid fever, properly belongs in this place. In addition to the usual means of differentiation, they made use also of media upon which typhoid bacilli had already grown, and in which the organisms isolated by them failed to develop. Furthermore, they applied to them the serum-reaction test. They examined thirty-seven specimens of water at times during which typhoid epidemics were prevailing as well as in the intervals between such epidemics. In nine instances they found organisms presenting the characteristics of the typhoid bacillus, in only two of which the water came from places in which typhoid was prevalent. Thirteen specimens of earth and sand gave seven positive results. They found in five persons suffering from leukæmia, acute tuberculosis without intestinal lesions, commencing dysentery, or chronic malaria, organ-

isms showing all of the properties of B. typhosus. Kister found in water which was obtained from a suspected spring a bacillus showing a great similarity to B. typhosus, and which could be distinguished from the latter only by the serum reaction. In many of these instances it is extremely probable that the organism in question was none other than B. typhosus.

Bacillus Monadiformis.—Obtained by Messea from typhoid dejections in Naples, and by Tavel and Lanz from pus in a case of peritonitis. It is a short rod, actively motile, possessed of a single polar flagellum. In its growth it resembles the colon bacillus, and shows reduction powers similar to those of that organism; it fails, however, to produce indol or to coagulate milk. It acidifies milk whey only slightly. It ferments glucose, and to a less extent lactose. It has no action on saccharose. It is non-pathogenic for mice.

Bacillus Icterogenes.—Described by Guarnieri and Vincent, who obtained it from the liver and blood in acute yellow atrophy of the liver. Kruse and Pasquale have found a bacillus resembling it in the dejections of typhoid cases, while Legendre and Bosc have also obtained a similar organism, both in the blood and in the internal organs, from patients exhibiting disturbances in the gastrointestinal tract. The organism is motile, produces indol, ferments glucose and sometimes lactose, but is without action on saccharose. Milk whey is slightly acidified. The organism is pathogenic for mice and guinea-pigs. It is probably a pathogenic colon bacillus failing to ferment saccharose.

*Group of Capsulated Bacilli.**

Non-motile bacilli of medium size, occurring singly, in pairs, or in small groups, and showing little tendency to the formation of threads. Staining by Gram and Weigert is negative. Occasionally in cultures and almost always in the animal body the organisms show a distinct tendency to become surrounded with a gelatinous membrane or capsule. All these organisms are closely allied to the colon group, differing from the members of the latter chiefly in the greater thickness of the individual organisms, in the more uniform development of the

*Besides the capsulated bacilli described in this chapter Migula enumerates the following additional varieties: B. capsulatus (Pfeiffer), highly pathogenic for white and house mice. Bacterium canalis (Mori), obtained from canal water; pathogenic for mice. B. Nicolaieri, obtained from a case of purulent nephritis, pathogenic for mice. B. Chinense (Hamilton), cultivated from Chinese ink; highly pathogenic for white mice. B. endocarditidis (Weichselbaum), obtained from a cardiac thrombus and infarcts of the spleen and kidney of a man; pathogenic for rabbits. B. pseudokeratomalaciæ (Loeb), obtained from a case of keratomalacia infantum; pathogenic.

mucigenous capsule, and the complete absence of motility. Certain cultural differences between the two groups are also observed, the chief one being the more abundant growth of the capsulated organisms, and in gelatin stabs the characteristic nail-like figure produced by them. This group of organisms is widely disseminated in nature, being present also in the animal body upon the oral and nasal mucous membrane and in the intestine. Some varieties are concerned with definite pathological processes.

Bacillus Aerogenes. *Synonyms.*—Bacterium lactis aerogenes (Escherich), Milchsäure Bacillus, Bacillus pyogenes, Bacterium aceticum, etc. It was first isolated by Escherich, who found it to be a normal inhabitant of the intestine of sucklings, especially of the upper part. Later it was repeatedly found in the fæces of adults, in sour milk, in cheese, in the air, and in water.

Morphology and Cultural Properties.—Non-motile, short rods with rounded ends, some of which are so short as to appear coccus-like. They vary from 0.5 to 1 μ in width, and from 1 to 2 μ in length. They occur singly, in pairs, and sometimes in threads up to 6 μ or even longer. They do not stain by Gram's method. They are asporogenous.

Gelatin.—The deep colonies are round, granular, and of a grayish-brown color; the superficial ones are larger, of a porcelain-white color, the centres being granulated and the whole colony appearing soft, slimy, and opaque. The gelatin stab gives a growth resembling a nail with a round head. The isolated colonies in the depth attain to a considerable size and are white. In old cultures the medium sometimes assumes a brownish coloration. There is no liquefaction.

Agar-agar.—The plate colonies are larger and somewhat more opaque than in gelatin; otherwise their properties are similar. The nail-like growth is not so distinct in the stab as in the previous medium.

Bouillon.—Bouillon becomes cloudy on the surface and a slimy membrane forms, while the bottom of the tube is occupied by a stringy sediment.

Potato.—Growth is abundant, yellowish-white in color, succulent; gas bubbles often form upon the surface. The cultures develop a cheese-like odor.

Milk is quickly rendered acid and coagulated. The coagulum is often torn apart by the rich gas formation.

Fermentation.—The several sugars are quickly fermented, and the gas formation in gelatin and agar depends upon the presence of sugar in the media. The acids formed from sugars are chiefly acetic, lactic, and formic, the gases consisting of carbon dioxide and hydrogen.

Opinions are divided with respect to the production of indol, some authors (Wilde) denying such a property to the organism. On the other hand, undoubted specimens of this organism produce indol after several days' growth in the thermostat.

One of the chief functions of this bacillus is the production of spontaneous coagulation in milk. The organism gains entrance into the intestinal canal of human beings or animals with cow's milk, and perhaps through the air.*

Pathogenicity.—Its most marked pathogenicity is shown in association with inflammations of the bladder and kidneys, as the cause of purulent cystitis and pyelo-nephritis, in which case the urine has a slightly acid reaction. The organism has been described under a variety of names. A similar bacillus has been found in the lungs, in hypostatic congestion, and once in a case of general infection in a human being associated with cholecystitis (Flexner). In experimental animals large doses are necessary to cause death, which even then is not to be attributed to the multiplication of the organisms so much as to the toxin production. Subcutaneous injections into rabbits produce localized suppuration, while intraperitoneal inoculation in guinea-pigs and mice causes a fibrinopurulent peritonitis, with a moderate number of the bacilli present in the blood, and a marked enteritis. The bacilli in the peritoneal exudate are usually somewhat longer than in cultures and are often surrounded by a typical capsule. Cultures injected into the urinary bladder give rise to cystitis, provided the urethra is ligatured in order to cause damming back of the urine. The bacilli gain entrance to the bladder either upon catheters or directly by absorption from the intestine (Posler, Lewin).

Bacillus Pneumoniæ. *Synonym.*—Pneumobacillus of Friedländer.

This capsulated bacillus was described by Friedländer in 1883, who ascribed to its action lobar pneumonia in man. According to Weichselbaum, it is the cause of this disease at most in a small percentage of cases. It is found frequently in the mucous membrane of the mouth and the air passages, as well as in the atmosphere. Bacillus pneumoniæ may serve as the type for a large number of similar capsulated and pathogenic bacilli which have been described within the last few years.

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* B. aerogenes is subject to pronounced variation in a direction which would tend to cause it to be confused with B. coli. The differential diagnosis is sometimes very difficult. The most important points are, probably, the immobility, the rapid coagulation of milk, the formation of gas, and the growth upon potato. Indol production, which hitherto has been considered a point of differentiation, is not reliable, although in general the aerogenes produces less indol and acts less constantly in this respect than do members of the colon group proper.

Morphology and Cultural Properties.—In morphology and cultural properties this bacillus is closely related to B. aerogenes. Old gelatin cultures of the former, however, show a marked brown discoloration on the medium. Further differences are found in its action upon milk, which is not coagulated except occasionally and by certain specimens of the organism. Lactose and glucose are fermented, although gas and acid formation are distinctly less than with B. aerogenes. The acids formed upon fermentation are acetic, formic, and succinic. Alcohol is also said to be formed. In growths upon potato and gelatin, according to Fermi, a diastatic ferment is produced. Indol is not formed. As obtained from the animal body and

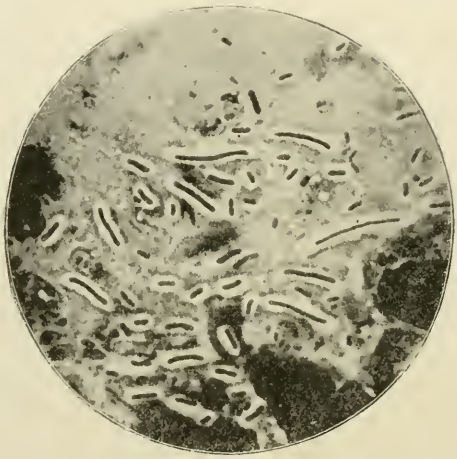

Fig. 17.—Friedländer's Bacillus in Pneumonic Sputum. × 1,000. (Fraenkel and Pfeiffer.)

at times from gelatin and other cultures the bacilli are provided with distinct, readily staining capsules.

Pathogenicity.—Netter found the organism in four and one-half per cent. of cases examined in the normal sputum of man; Besser obtained it several times from the normal respiratory passages, and Pansini isolated it from tuberculous sputum. The belief expressed by Friedländer that this organism was the cause of croupous pneumonia, has been shown through the studies of Weichselbaum and many others to be erroneous. It is, however, not infrequently concerned with the production of lobular inflammations of the lung, and has been found in secondary lung abscess following lobar pneumonia (Flexner), and in abscess of the lung (Cohn). Sydney Wolf isolated it from cases of empyema, and it has been found in other diseases, such as pericarditis, parotitis, otitis media, angina, endocarditis, ulcer of the cornea, pyelonephritis, cystitis, and angiocholitis, as well

as in a suppurating hæmatoma of the scrotum. From such local developments septicæmias have resulted. Small animals, mice, guinea-pigs, and rabbits are susceptible to its action. Subcutaneous inoculation in mice will cause death in from twenty-four to forty-eight hours, with the production of general infection. Special localizations in the serous membranes may occur. Similar inoculations in rabbits often produce localized abscesses, whereas intraperitoneal inoculation is more apt to cause fatal infection. The same is true for the guinea-pig.

Bacillus Ozænæ. *Synonyms.*—B. capsulatus mucosus (Fasching), B. mucosus ozænæ (Abel).

This organism was at first confounded by Thost, Babes, and Hajek with Friedländer's bacillus. The distinctions were pointed out by Abel and Löwenberg. A careful comparative study has been made by Wilde (1896).

Morphology and Cultural Properties.—In morphology the organism resembles B. pneumoniæ. It decolorizes by Gram, and is non-motile. The chief differential points are brought out in the cultures. The colonies are more transparent, softer, and more mucoid than is the case with the other organisms. In stab cultures the nail-like form with rounded head is less pronounced on account of the tendency of the growth to spread over the entire surface. Again, this organism is also more susceptible to external influences. Growth ceases at temperatures about 15° C., and strongly alkaline and acid media are not suitable for its development. It dies more quickly on potato. Milk is not coagulated, and gas is only rarely formed in sugar media, and then in minimum quantities. Lactose bouillon is rendered very slightly acid, in all probability through action on the minimal quantities of a second carbohydrate, and not upon the lactose itself. Growth on potato is colorless and slime-like, and only rarely are gas bubbles included. Indol is not formed.

Its chief importance attaches to the regular occurrence of this organism in ozæna. In plate cultures from such cases it is found, when not present in pure cultures, to be the predominating organism. A somewhat similar bacillus, it must be admitted, has been isolated from cases of purulent ulceration of the nasopharynx, purulent rhinitis, and syphilitic coryza. Mice are very susceptible to inoculation, being killed, according to Abel, by minimal subcutaneous injections. The bacilli are abundant in the blood. Guinea-pigs resist subcutaneous inoculation, but die after intrapulmonary and intraperitoneal injections. The exudates of the serous cavities present a slimy appearance. Rabbits are relatively resistant.

Bacillus Rhinoscleromatis.—First demonstrated by von Frisch

in microscopic sections of the nasal tumor, and cultivated by Paltauf and von Eiselsberg. This organism was also included in the comparative studies of Wilde.

Morphology and Cultural Properties.—The morphology and cultural characteristics are, according to Wilde, largely the same as those of B. ozænæ. One point of distinction is the complete absence of capacity to ferment the sugars, although its growth upon potato at high temperatures is said to be followed by the appearance of gas bubbles. Upon potato it is usually not distinguishable from the preceding organism, although sometimes a brownish and opaque coat may be developed. In cover-slip preparations and in tissues hardened in alcohol the organism decolorizes by Gram.

Pathogenicity.—The bacilli are, according to Kruse, characteristic for the lesions of rhinoscleroma, from which they may be readily and regularly obtained in cultures, often without the admixture of other organisms. In the pathological condition, which is of the nature of the granulation tumor, the organisms appear in masses, usually embedded in the slimy material which they have formed. Sometimes they appear singly in the swollen cells (cells of Mikulicz) surrounded by capsules. The manner of their arrangement in tissues, as well as their constant presence, offers, of course, strong presumption that this organism stands in a causal relation with the pathological process. Stepanow inoculated pure cultures into the anterior chamber of the eye of guinea-pigs, producing a granulation tissue suggestive of the morbid process in human beings. The pathogenicity for animals differs only in degree from that of Friedländer's bacillus and the ozæna bacillus. Guinea-pigs and rabbits can be killed by injecting large doses intraperitoneally, but only small numbers of the bacilli are found in the blood.

Differential Diagnosis.—Friedländer's bacillus, B. ozænæ, and B. rhinoscleromatis are difficult to separate from one another. The slight differences in cultural properties scarcely suffice, inasmuch as there are intermediate transitions in colony formation, and even in action upon milk and sugars. Attempts have been made to differentiate the organisms by means of Pfeiffer's reaction—in Wilde's hands with negative results, probably because most of his rabbits died of marasmus. The serum of one rabbit which had been immunized to B. rhinoscleromatis was active also against Friedländer's bacillus, whereas it showed less action against B. ozænæ. Kraus, on the other hand, has found that subcutaneous injections of dead rhinoscleroma bacilli produce the capacity of agglutination in the blood serum, this blood, however, having no action on Friedländer's bacillus. On the other hand, animals inoculated with dead Friedländer's bacilli give a

serum which is active for B. rhinoscleromatis. These results are still so doubtful as not to justify us in passing finally upon the question of identity or difference in regard to these organisms.

Bacillus Capsulatus Septicus. *Synonym.*—B. proteus hominis capsulatus, Bordoni-Uffreduzzi.

First described by Bordoni-Uffreduzzi, Banti, and Bonome, and probably identical with a bacillus also obtained by Howard in the United States. These organisms have been isolated from a variety of general infective processes in human beings. They agree in many respects with Friedländer's bacillus. They differ, however, in that in some instances the organisms in sections of hardened organs were found by Bordoni-Uffreduzzi to stain by Gram's method. In cultures, moreover, the short individuals are refractory to Gram's stain, whereas the longer threads decolorize easily. Howard, who obtained his organism from a case of chronic cystitis, pyelitis, and pyelonephritis, found that the organism was pleomorphic, grew very luxuriantly on potato, was a rapid gas-producer, but did not decolorize by Gram's method. This group of bacilli is distinguished from the pneumobacillus of Friedländer by a greater virulence. Minute quantities of cultures of the blood of animals dead of the disease are fatal in subcutaneous inoculations of mice. Somewhat larger doses kill rabbits and guinea-pigs. Howard mentions pathogenicity for both rabbits and guinea-pigs. By repeated cultivations on artificial media the virulence is gradually lost, in which cases the only remaining differential point as compared with Friedländer's bacillus would be the reaction to Gram's stain.

Group of B. Enteritidis.

All these various bacilli are closely related to B. typhosus and B. coli communis, so that they may be considered as constituting an intermediate group of microorganisms. The type of this group can be chosen indifferently in B. enteritidis of Gärtner, or in the hog-cholera bacillus of Salmon and Smith. On account of the wide distribution of the hog-cholera bacillus, and because of the fact that in this country its properties have been so well worked out and established, this organism could have been selected with greater propriety than that which supplies the name to the group, were it not for the fact that German writers have so hopelessly confused the hog-cholera bacillus with the swine-plague bacillus—a totally different organism belonging to the hemorrhagic septicæmia group of bacilli—that for the present at least an extrication from such confusion can scarcely be hoped for.

Bacillus Enteritidis.—Obtained by Gärtner from the flesh of a cow which had been slaughtered because of disease of the intestine, and from the spleen of a man who had succumbed to infection after eating the flesh of this animal. Similar organisms have been described by Karlinski, who obtained it from a non-fatal case of meat-poisoning; by Lubarsch, whose organism came from the organs of a child supposed to have died from Winckel's disease; and by Durham from the liver of a person dead of meat-poisoning in an epidemic occurring in Chatterton in England. Closely related, if not identical, are several bacilli obtained from meat-poisoning by Van Ermengem and Känsche, Gaffky and Paak, etc. (pages 662 and 663).

Morphology and Cultural Properties.—Short, thick, motile bacilli, in part surrounded by capsules, and often staining irregularly. Gram's stain is negative. Asporogenous. In plate cultures the colonies are pale gray in color, translucent, round in form, suggesting the colonies of B. aerogenes. On potato there is a grayish-white to grayish-yellow, shining, membranous-like growth. The growth on gelatin or agar-agar is very similar to that of B. aerogenes. According to Petri, indol is not formed, but it is highly probable that eventually this substance appears in the cultures, although it may be absent the first twenty-four to forty-eight hours. The statement by Lubarsch that milk is coagulated is evidently erroneous. The characteristic appearance produced in milk is due to an alkali which may succeed a transient acid stage when the organisms grow in contact with air. After a few days the milk medium assumes a further characteristic opalescent appearance due, according to Cushing, to the solution, through the action of the alkali, of the fatty membranes surrounding the casein molecules. Gas is formed in dextrose, but not in lactose or saccharose. The minute bubble of gas which appears in the fermentation tubes in lactose media is probably due to the accidental inclusion of a second carbohydrate, possibly dextrose (Cushing).

Pathogenicity.—The frequent occurrence of the organism as the cause of meat-poisoning is sufficient evidence of its pathogenicity for man. In the organs of individuals who have succumbed, organisms were found in smears or in the sections of various organs. The flesh of animals containing this bacillus is poisonous even after cooking. The period of incubation in human beings after the ingestion of such flesh varies from twenty-four hours to seven days. Mice, guinea-pigs, rabbits, pigeons, goats, and lambs are susceptible to experimental inoculation, while dogs, cats, rats, chickens, and sparrows are refractory. Mice and guinea-pigs succumb not only to subcutaneous injection, but also to feeding with the microorganism. The bacilli

are found in groups in the infected organs just as has been noted for the colon and typhoid bacilli. In animals poisoned with the organism there are intense enteritis, swelling of the follicles, and sometimes intestinal hemorrhages.

Bacillus of Hog Cholera. *Synonym.*—B. choleræ suis. First clearly described by Theobald Smith in 1886. Studied and differentiated from the bacillus of swine plague and other organisms of infectious swine diseases by Th. Smith, Welch and Clement.

Morphology and Cultural Properties.—The bacilli of hog cholera appear as short rods with rounded ends, averaging from 1 to 2 μ in length and about 0.6 μ in breadth. Longer or shorter forms may occur. The bacilli are actively motile and grow readily on all the ordinary culture media at temperatures between 30° and 38° C. They do not liquefy gelatin. The growth on gelatin and on agar-agar has a grayish or whitish color, often with a bluish translucence. Bouillon cultures present a diffuse cloudiness with a whitish sediment, but without a surface membrane. The growth on potato assumes generally a brownish or yellowish tint, but it may be white, and sometimes is indistinct, even when microscopically the growth is abundant. Bacilli are killed by exposure for ten minutes to a temperature of 58° C. In cover-slip preparations from fresh juices and tissues of animals dead of hog cholera the bacilli stain readily, and for the most part uniformly, with the ordinary anilin stains. If stained specimens be treated with acetic acid many bacilli appear with a clear centre and stained margin, which may be either uniform or slightly thicker at the poles. Polar staining is sometimes observed. Various irregularities in staining appear in old cultures. The growth in milk is the chief cultural characteristic. There may be a transient acidity which is followed by alkalinization, the development of an opalescent appearance, and the formation of a conspicuous bluish ring at the surface of the medium. Dextrose is fermented. The organism has no action on lactose, although in lactose bouillon free from muscle sugar a bubble of gas may form upon the surface, owing to the presence of a second carbohydrate in the original milk-sugar.

Pathogenicity.—In nature it most commonly occurs in a disease in hogs. The lesions in the intestines spoken of as "buttons" are the most characteristic. Scarcely less common, but much less typical than these, are diffuse and circumscribed areas of diphtheritic inflammation in the intestinal mucosa. Necrotic foci similar to these buttons may occur in various parts of the body besides the intestine, and small yellowish or yellowish-white or reddish necroses are common in the liver. More rarely areas of pneumonia are found in which the hog-cholera bacillus is present in the lungs. Not infrequently these

pneumonias are caused by the swine-plague bacillus (see page 688), or a mixture of the swine-plague bacillus and the hog-cholera bacillus (Welch and Clement). Hog-cholera bacilli are pathogenic for rabbits, mice, guinea-pigs, and pigeons. Inoculations succeed whether the injections be made subcutaneously, into the serous cavities, or into the blood. In fatal cases in rabbits necrotic patches and diphtheritic exudates are found in the intestinal mucosa, and numerous necroses in the liver. The bacilli tend to grow in clumps, and may be obtained from the diseased organs and in smaller numbers from the blood.

Bacillus Icteroides. *Synonym.*—Bacillus of yellow fever. Discovered by Sanarelli in 1896 in the cadavers of persons dead of yellow fever, and believed by him to be the cause of this disease.

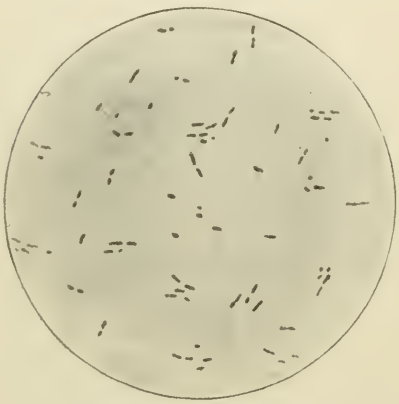

Fig. 18.—Bacillus Icteroides. (Sanarelli.[20])

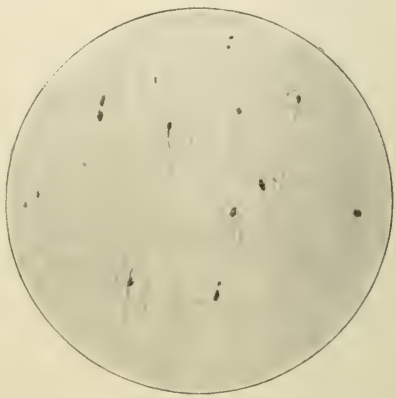

Fig. 19.—Bacillus Icteroides, showing Flagella. (Sanarelli.[20])

Morphology and Cultural Properties.—The bacillus appears in slender, rod-shaped forms, 2 to 4 μ in length and one-half to one-third as wide. In cultures it shows distinct polymorphism. Thread-like and swollen involution forms are common in old cultures. In artificial cultures it appears chiefly as a diplobacillus, occurring in the organs in small groups. It is easily stained with the ordinary anilin dyes. Sometimes the centre of the organism contains an unstained spot. Gram's stain gives negative results. The bacillus is asporogenous, motile, possessing from four to eight long flagella. It is a facultative anaerobic. It grows on the ordinary culture media readily. Colonies on gelatin are round or kidney-shaped and granular. It causes no liquefaction. On agar incubated at 37° C. the colonies are round, grayish, and faintly iridescent. Cultivated at temperatures of 22° to 26° C. on this medium they are opaque, shining, and

milk-like. On potato there is a fine transparent growth. Bouillon is diffusely clouded. There are no precipitate and no membrane. A small amount of indol and acid are produced. According to Sanarelli lactose is but little affected, whereas glucose and saccharose are fermented more strongly. The most characteristic appearance is in litmus-milk, and is similar to that found with the bacillus of hog cholera. Cushing, who has made a careful comparative study of this group of bacteria, finds that the bacillus icteroides agrees with the preceding members in that it actively ferments dextrose, but is without effect upon lactose or saccharose, excepting in so far as the last-mentioned sugar in commerce usually contains a second carbohydrate. The similarity in cultures exhibited by this bacillus to the hog-cholera bacillus has been pointed out by Reed and Carroll, who from this and their experiments upon animals consider that the two organisms may be identical.

Pathogenicity.—The conditions under which the organism has been found in human beings have led Sanarelli to regard it as the cause of yellow fever, for which assumption the evidence is as yet insufficient. Most experimental animals are susceptible. Birds, however, seem to be refractory. Continued cultivation outside of the body is associated with a loss of virulence. Injections subcutaneously, into the circulation, or into the serous cavities cause the death of mice, guinea-pigs, and rabbits. Intravenous injections into dogs are also followed by characteristic results. In the last-named animal bloody vomiting, hæmaturia, albuminuria, hemorrhagic gastroenteritis, jaundice, and extensive fatty degeneration of the liver have been noted. Necroses in the liver, similar to those which appear in this organ in animals inoculated with the bacillus of hog cholera have been observed in the dog, guinea-pig, and rabbit (Reed and Carroll).

Poison Production.—B. icteroides produces a virulent poison. Filtered bouillon cultures give rise in rabbits, guinea-pigs, cats, dogs, goats, asses, and horses to symptoms of intoxication similar to those following injections of the cultures. The symptoms in the dog are the same as those following intravenous injections of the culture. Sanarelli had the temerity to inject filtered cultures into human beings in whom he claims to have produced the typical disease-picture of yellow fever.

Immunization.—With repeated and regularly increasing doses of the poison it has been possible to immunize various animals, including the horse. The serum obtained from these immunized animals has generally shown distinct protective and healing action in experimental investigations. This serum has also been applied to human beings, but thus far without marked benefit. An agglutination reac-

tion towards this bacillus has been found in the blood of persons suffering from yellow fever.

Bacillus Paracolon.—First described by Widal, in 1897, who obtained it from an œsophageal abscess occurring some years after an attack of typhoid fever. With this organism he grouped several others having similar characteristics, such as Bacillus psittacosis of Nocard, and the microbe of septicæmia in the calf described by Thomassen. A similar organism was isolated from the blood of a patient believed to be suffering with typhoid fever by Gwyn. In Gwyn's case the blood serum of the individual agglutinated this organism, but had no effect upon B. typhosus. Recently Cushing has isolated from an abscess of the rib, which appeared during convalescence from a prolonged fever of the enteric type, a similar organism agglutinating in the same way with the blood of the patient, the blood having no effect upon B. typhosus.

Morphology and Cultural Properties.—The organism described by Widal is a motile bacillus, decolorizing by Gram, which fails to liquefy gelatin, produces a clouding in bouillon but no membrane. It grows on potato as a yellowish-green film, and forms a small amount of gas in glucose agar. The two essential points, in Widal's opinion, which distinguish it from the colon family are the absence of indol production and of fermentative action on lactose. Furthermore, the organism fermented mannite. Like the organisms just mentioned, it presented a strong agglutinating action with the patient's blood. Gwyn states that his organism also fermented saccharose. Cushing's studies, which were made with the view of testing the fermentative properties of these several organisms, and the paracolon bacillus isolated by him, indicate that neither Gwyn's bacillus nor his own is capable of fermenting any other sugar than glucose. The most characteristic reaction is found in milk, in which it produces early alkalinization, with the subsequent opalizing modification. On potato Cushing's bacillus and the paracolon bacillus of Gwyn behave like B. typhosus.

Pathogenicity.—The conditions under which Widal's and Cushing's paracolon bacilli were obtained, of course, speak for the pathogenic character for human beings of this organism. The case is not so clear with Gwyn's bacillus. He reports no animal experiments, and the mere fact of isolation from the blood, while suggestive of pathogenicity, might certainly be regarded as an accidental occurrence, were it not for the fact of the positive serum reaction. Cushing injected his organism into mice, guinea-pigs, and rabbits. Mice succumbed to large doses, and the bacilli were found in the organs and the blood. A guinea-pig inoculated subcutaneously with a loop of a

solid culture developed a local swelling, from which the animal finally recovered. Large doses of cultures injected intravenously into rabbits produce an acute illness, and sometimes local lesion at the point of injection, from which the animals finally recover.

Immunization.—The absence of marked pathogenic properties for animals makes the question of immunization of little moment. A more important point for consideration, however, presents itself in the agglutination phenomenon which follows recovery from injections into the rabbit. The serum of animals treated in this way acquires clumping properties of high degree. Cushing gives an instance in which active clumping occurred in dilutions of from one to five hundred. He also mentions an instance in which injections of the bacillus of hog cholera into such an immunized rabbit failed to kill the animal, although a control animal died at the end of a few hours from a similar injection. The animal which survived also developed agglutinative properties as regards B. choleræ suis. Cushing is of the opinion that the chief differential point is pathogenicity, which serves to separate B. enteritidis and the bacillus of hog cholera from the other members of the group, while remaining examples are to be distinguished by the serum reaction. This reaction can also be utilized for separating the two first-mentioned organisms from other members of the group. Durham goes so far as to consider that bacilli of this general group have probably been mistaken hitherto for the typhoid bacillus, particularly in cases presenting the clinical features of typhoid fever, in which intestinal lesions have not been found, but from which microorganisms not distinguishable from the typhoid bacillus by the ordinary methods have been cultivated. Without discussing this somewhat sweeping and unsupported view, it is more than likely that he is correct in his statement that the typhoid bacilli of certain authors which have the power of gas production certainly belong to this so-called intermediate group.

Bacillus Typhi Murium. *Synonym.*—Bacillus of mouse typhoid. Isolated by Loeffler during a spontaneous epidemic in mice, occurring in the Hygienic Institute at Greifswald.

Morphology and Cultural Properties.—Actively motile rods, resembling the typhoid bacilli, which sometimes grow into filaments. Not stainable by Gram's method. The colonies in the depth are small, round, finely granular, yellow to brown in color, while on the surface they are spreading with irregularly indented edges and delicate furrows. They resemble typhoid colonies, excepting that they are more granular and grow more rapidly. Stab cultures show a nail-like growth, the head being flat in form. On potato a white, moderately abundant membrane forms, while the surrounding sub-

stance assumes a dirty bluish-gray color. In glucose-bouillon gas is formed. According to Cushing other sugars are not fermented. Milk is not coagulated, but an alkaline reaction is developed. No statement is made regarding indol production.

Pathogenicity.—The bacilli are very virulent for white and gray house mice and for field mice. Subcutaneous injections and feeding are both effective. The bacilli are found in various organs, but are especially numerous in the liver. They appear in clumps inside the capillaries, surrounded by dead liver cells and emigrated leucocytes. The spleen is swollen. Feeding causes death in from seven to fourteen days with the picture of hemorrhagic enteritis. The mesenteric glands are swollen, and the organs contain bacilli. Other laboratory animals (guinea-pigs, rats, pigeons, small birds) do not react to the ingestion per os, but succumb in from two to ten days to subcutaneous inoculation. Rabbits react only with a local focus of suppuration. Mereschkowsky has described a similar bacillus which produced an epidemic among marmots. It is virulent in a similar way to the bacillus of mouse-typhoid for house and field mice. These organisms are closely related to the meat-poisoning bacilli (see below), but are to be distinguished from them by feeding experiments.

Bacillus Fæcalis Alcaligenes.—Described by Petrushky, who obtained it in a number of instances from fæces, and who regarded it as resembling B. typhosus. Consists of actively motile bacilli, supplied with flagella. Asporogenous. Negative to Gram's stain. Growth on gelatin similar to that of B. typhosus. Milk is not coagulated; alkali is produced. It is said not to ferment sugars, and to grow on potato with the production of a moderately thick brownish membrane. Germano and Maurea state that the organism may produce slight acidity in milk. This feature has been noted as the first stage in many of the other organisms of this group. It shows no reaction with the serum of typhoid cases.

Bacillus Breslaviensis. — Isolated by Van Ermengem and Känsche, who regarded it as the cause of two epidemics of meat-poisoning in Morseele and Breslau. The bacilli are asporogenous, from 0.6 to 1.5 μ long, and about one-third as thick. They are actively motile, possessing four to twelve long flagella. They are not stainable by Gram. Cultures are in general like those of B. coli. Milk, however, is said not to be coagulated. Dogs and cats are immune. Mice, pigeons, and rabbits respond both to feeding and to inoculation. Cooked cultures and cooked infected meats are still poisonous. The symptoms produced are enteritis, paralysis, and convulsions. The differential diagnosis as compared with the colon bacillus is found in the absence of indol production and milk coagulation, and the pres-

ence of toxic and pathogenic properties. The bacillus shows relations with B. enteritidis in that the poison is not killed at the boiling temperature, a fact which also brings it in agreement with the next two bacilli to be described.

Bacillus Friedebergensis.—Obtained by Gaffky and Paak from sausage which had given rise to extensive poisoning. The bacilli are moderately motile, appearing in rods twice as long as broad, and sometimes in the form of long threads; they are asporogenous, and do not stain by Gram's method. The cultural properties are intermediate between those of the aerogenes and colon group. The organism is capable of development at the temperature of the ice-chest, and resists drying for months. It does not produce indol, and fails to coagulate milk. It is highly pathogenic for mice, guinea-pigs, and rabbits. Fed to guinea-pigs, mice, and monkeys, it is highly virulent. It is less poisonous for dogs, young cats, and rabbits, and not at all for swine. Intestinal symptoms occur whether the organisms are introduced under the skin or into the intestinal canal. The distinction from B. coli depends upon the absence of indol production and milk coagulation, and from the typhoid bacillus by certain cultural and morphological properties, as well as pathogenicity. The poison is destroyed at the boiling temperature, a point which distinguishes it from B. breslaviensis.

A similar organism, although lacking in pathogenic properties, was found by Gaffky and Paak in the intestinal contents of animals, and once in the cadaver of a mouse which had been kept in the earth. From the latter source it possessed virulence.

Bacillus Morbificans Bovis.—Obtained by Basenau from the flesh of a cow which had suffered from puerperal fever and been killed. Highly pathogenic for mice, rats, guinea-pigs, and rabbits, but not pathogenic for dogs and cats. Feeding gave positive results in susceptible animals. It is considered probable that an instance of meat-poisoning from eating flesh derived from animals with puerperal fever may have been due to this organism. Sterilized cultures are not pathogenic. The differential diagnosis from B. coli communis depends upon its behavior in milk, which is not coagulated, and its slight capacity to ferment grape sugar. The organism appears to be closely related to B. friedebergensis.

Group of Proteus Bacillus.

Bacilli of medium size, asporogenous, showing variable behavior with Gram's stain; aerobic or facultative anaerobic. Colony formation for the most part is characteristic, in that a tendency to migration

in the depth and upon the surface of the culture media is exhibited. Some of the forms are distinctly putrefactive. The organisms are sometimes described as pleomorphic, the pleomorphism consisting in the appearance of shorter and longer rods, the former sometimes approaching cocci in size. This condition is more especially observed in old cultures. More rarely spiral-like figures are formed. Gelatin is quickly liquefied by some, more slowly by others, while still others are incapable of peptonizing this substance.

Bacillus Proteus Vulgaris (Hauser). *Synonyms.*—Bacterium vulgare (Lehmann and Neumann), B. proteus hauseri, B. albus cadaveris (Stricker and Strassmann). Obtained by Hauser from decomposing animal substances, infusions, and gangrene. Closely related, perhaps simply varieties, are Proteus mirabilis and P. zenkeri. The extremes are formed by P. vulgaris, which is an active liquefier,

Fig. 20.—Bacillus Proteus Vulgaris Hauser, from a Pure Agar Culture. × 800. (Lehmann and Neumann.)

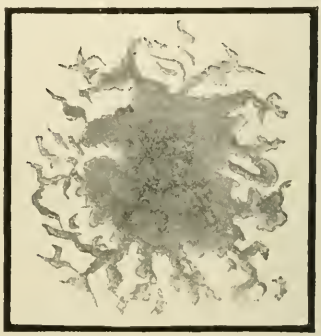

Fig. 21.—Bacillus Proteus Vulgaris. Deep-seated colony on gelatin plate. × 60. (Lehmann and Neumann.)

and P. zenkeri, which does not liquefy gelatin. Transformations have been observed in artificial cultures by Hauser.

Morphology and Cultural Properties.—The bacilli vary widely in size. They average about 0.6 μ in width, being from 1.2 to 4 μ in length. Very short forms and very long threads, however, are also observed. The organism is actively motile, and supplied with flagella, which may, on long rods, amount to one hundred or more in number. In young cultures Gram's stain is sometimes positive; in older cultures negative. Some individuals under both conditions may retain the stain, while the others are decolorized. The most characteristic colony appearances are obtained in weak gelation media (about five per cent.). Growth takes place at room temperature, but more rapidly in the thermostat at 22° to 24° C.; it is still demonstrable in the ice chamber. The young growths are granular, pale yellowish in color, and under the low powers of the microscope may be seen to send out projections into the surrounding medium. In

rapidly liquefying varieties there soon appears a peripheral depression, in which actively motile bacilli are seen. These bacilli penetrate the medium from the periphery, giving rise to the peculiar anastomosing lines and secondary growths, denominated "swarming" colonies. The colonies which develop on the surface of the medium are sometimes spreading, and thinner and more translucent than those in the depth. A similar tendency to migration is observed and adjacent colonies tend to coalesce. Similar swarming is observed in non-liquefying varieties, and a peculiar lace-like network in the

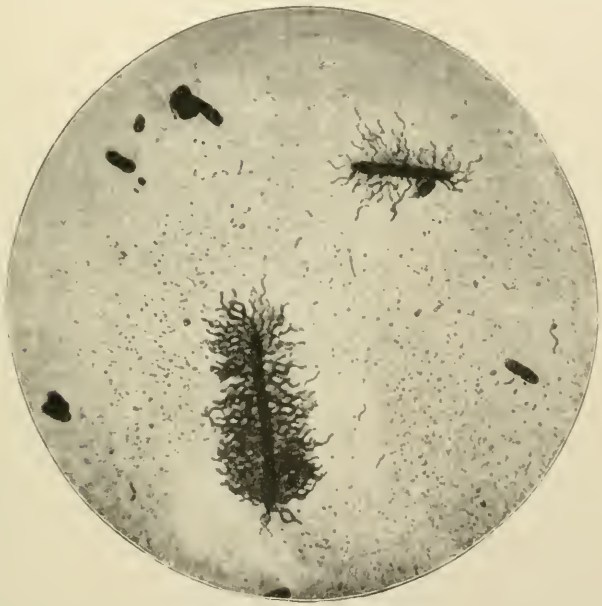

FIG. 22.—Bacillus Proteus Vulgaris, Showing Flagella. × 1,000. (Fraenkel and Pfeiffer.)

media, extending throughout the plate, is met with. Under the microscope characteristic convoluted, irregularly swollen and sausage-like appearances come into view. Stab cultures in gelatin show similar peculiarities, except that, the growth being solid and continuous, swarming takes place at right angles, with the result that hair-like projections into the media along the line of the stab are produced. The more concentrated the medium the slower is the liquefaction, and the less marked the migration. Growth takes place on agar in the form of a soft, moist, thin, transparent membrane. Migration is more or less confined to the moist surface of the medium. Growth takes place abundantly on potato, producing a soft, grayish-white membrane. Upon blood serum there is imperfect peptonization, and upon this medium the growth is more rapid at the temperature of the

thermostat. The bacilli grow more extensively in the presence of air. Peptonization does not take place in an oxygen-free atmosphere. Sugars are fermented with the exception of lactose (Roger, Th. Smith). Milk is rendered slightly acid and coagulated, the coagulation, however, appearing sometimes only after many days or weeks. Coagulated milk is afterwards liquefied (Lehmann and Neumann). Disagreeable putrefactive gases are formed on the ordinary media, especially in milk. Non-albuminous media are adapted to its growth. Indol, phenol, and H_2S are evolved, and nitrates are reduced to nitrites and in part to ammonia (Petri, Lewandowski).

Pathogenicity.—The proteus bacilli show a very variable action towards experimental animals, some varieties in ordinary amounts causing slight or no symptoms. Larger quantities, however, may produce subcutaneous abscesses, or after introduction into the circulation or the serous cavities bring about death with symptoms of intoxication. The organisms for the most part do not increase in the bodies of these animals unless necrotic tissue is present, in which they can find a nidus for multiplication. In human beings mixed infections with pyogenic cocci have been reported. Hauser observed them in gangrenous and purulent peritonitis, puerperal endometritis, and gangrenous phlegmon of the hand. Similar instances have been reported by Brunner and Charrin, who attributed a gangrenous pleuritis to such a mixture. In the severest of these mixed infections the symptoms were usually referable to intoxication and, as a rule, no increase of bacilli in the body generally was observed. E. Levy, in studying an epidemic of hemorrhagic diarrhœa, found examples of poisoning through the ingestion of meat infected with B. proteus. The intestinal contents and fæces contained many proteus bacilli, although, as a rule, no invasion of the body had taken place. Examples of pure proteus infection have also been reported—one by Flexner, in which this organism was the cause of a purulent peritonitis. A general infection has been described by Foà and Bonome following infarction of the intestine, while Flexner has observed a similar condition associated with abscess of the brain and the prostate gland, no other microorganism being present. In some instances of general infection ascribed to this bacillus the investigators have not shown beyond doubt that the invasion had occurred during life. Cystitis and pyelonephritis have been attributed to it (Krogius, Schnitzler, Schmidt, Aschoff). Ohlmacher has described the organism as the causative agent in cerebrospinal meningitis. Kühnau has drawn attention to mixed infection with B. proteus in diphtheria of the throat, and del Vecchio has described three cases of hospital gangrene in which the pyogenic cocci and proteus were combined. Charrin

and Nobécourt observed the frequent presence of Proteus vulgaris in cases of gangrene of the lung.

De Nittis has rendered guinea-pigs immune to proteus infection by intraperitoneal injections of cultures, and from these animals he obtained a serum capable of protecting rabbits from fatal doses of the organism. The filtered cultures are toxic. Schmiedeberg's sepsin, obtained from putrefying yeast, acts in precisely the same manner as the metabolic products of proteus and would seem to be a product of proteus development (Levy).

Several varieties of proteus bacilli differing in cultural or pathogenic properties from Proteus vulgaris have been described in association with pathological processes. These are:

Bacillus Proteus Septicus (Babes).—Obtained from the necrotic mucous membrane of the intestine and the internal organs of a child. The bacilli are pleomorphic, stain by Gram's method, rapidly liquefy gelatin and blood serum, and produce a pronounced putrefactive odor. The growth is similar to that of Proteus vulgaris. It is highly pathogenic for mice.

Bacillus Proteus Lethalis (Babes).—Obtained from a case of gangrene of the lung. Non-liquefying. Short, thick bacilli. Stain by Gram's method. Highly pathogenic for mice and rabbits, less so for guinea-pigs.

Bacillus Murisepticus Pleomorphus (Karlinski).—Obtained from pus of a phlegmon of the leg. Pleomorphic, asporogenous. Negative to Gram's stain. Colonies upon gelatin resemble those of Proteus vulgaris and P. mirabilis; they resist high temperatures. They are pathogenic for mice, producing a septicæmia. Rabbits are also susceptible.

Bacillus Proteus Fluorescens (Jaeger).—Obtained from cases of Weil's disease during life from the urine, and after death from the organs. Demonstrable in microscopical sections. Pleomorphic. Gram's stain negative. Actively motile, asporogenous. In culture media great variation is shown. On the same plate may be observed liquefying and non-liquefying, or very slowly liquefying, colonies. Sooner or later a greenish fluorescence appears. The cultures have a disagreeable odor. Sugars are fermented. On potato there appears a thick, pale yellow or delicate brown membrane, producing a bluish-gray discoloration of the medium. The organism is pathogenic for mice and also for pigeons. The bacilli are found in the organs and sometimes in the intestinal contents. B. proteus fluorescens is regarded by Jaeger as the cause of Weil's disease, the intestine serving as the portal of entry, and he would make a diagnosis from cultures obtained from the urinary sediment.

Bacillus Dysenteriæ Liquefaciens.—Obtained by Ogata from eleven cases of Japanese dysentery. The bacilli are actively motile, thin, often united in pairs. It stains by Gram, liquefies gelatin, and sends out short peripheral processes. It is pathogenic for mice and guinea-pigs by subcutaneous inoculation. Injections into the rectum of guinea-pigs and cats produce ulcers. The lesions in the internal organs from subcutaneous injections consist of nodules in the liver, spleen, and large intestine, which become caseous and disintegrate. This organism resembles the foregoing only in its cultural properties; in its morphology and pathogenicity it is totally different from the proteus group.

Bacillus Ulceris Gangrenosi (Sternberg).—Obtained by Babes from multiple ulcers in a case of prurigo, and from the internal organs from the same case after death. It is a motile bacillus, asporogenous, often staining irregularly with methylene blue, and decolorizes with Gram. Gelatin is liquefied with the production of an ochre-yellow sediment. Upon the surface of potato a brownish, bright membrane forms. Gas is produced in cultures, but no disagreeable odor is generated. Mice succumb to subcutaneous inoculation in about eight days. A small number only of the bacilli are present in the blood. Rabbits are also susceptible.

Bacillus Meningitidis Aerogenes.—Obtained by Centanni from two cases of meningitis. The bacilli are motile and occasionally occur in long threads. They are asporogenous and do not stain by Gram's method. Bouillon is clouded, and a grayish-yellow growth takes place upon potato. Blood serum is not stated to be liquefied. Subdural inoculations in rabbits cause death in a few hours, days, or weeks, according to circumstances, dose, etc. Infection may also be produced by the introduction of cultures into the nasal passages.

Bacillus Pneumoniæ Liquefaciens. *Synonym.*—Pneumobacillus liquefaciens bovis (Arloing). Obtained by Arloing regularly, although in small numbers, from the pneumonic exudate of cattle suffering from pleuropneumonia. Since the injection of this organism into the animals did not reproduce the typical disease, its etiological importance was questioned, and we now know, from the studies of Nocard and Roux, that it has no significance in the production of pleuropneumonia. The bacilli are non-motile, short, resembling cocci. They stain by the usual methods, and also according to Gram. They are asporogenous and quickly liquefy gelatin. Growth takes place upon the ordinary culture media. Subcutaneous inoculation of cultures in amounts varying from 1.5 to 2 c.c. cause abscesses in oxen. Larger quantities injected into the lungs or blood-vessels sometimes caused pneumonia and death. Guinea-pigs, dogs, and rabbits

are less susceptible, but react to intraperitoneal injections. The cultures quickly lose their virulence.

Group of Influenza Bacilli.

Small bacilli, asporogenous, non-stainable by Gram, appearing either singly or in pairs, more rarely in small groups. The individuals are either rod-shaped or slightly curved or bent. They are obligatory parasites, or present marked difficulties of cultivation outside the body.

Bacillus Influenzæ.—Discovered by R. Pfeiffer in the sputum of patients suffering from influenza and cultivated by him on special media.

Morphology and Cultural Properties.—The bacilli appear as non-motile, very small, somewhat plump rods, occurring singly or in pairs, staining with some difficulty with the ordinary anilin stains, and readily decolorized by Gram's method. They vary from 0.2 to 0.3 μ in width, and average about 0.5 μ in length. The organisms outside the body are very susceptible to injurious influences, such as drying, suspension in unfavorable media, for example water, while in bouillon they may retain their vitality up to two weeks. Pure cultures are obtained on the surface of agar-agar or blood serum previously smeared with a small amount of blood. The addition of leucocytes, from pus, for example, also renders the medium more favorable for the reception of the bacilli. At the temperature of the thermostat colonies develop at the end of from twenty-four to forty-eight hours, and appear as hyaline droplets, usually quite homogeneous and colorless. The older colonies sometimes show a yellowish or brownish centre, which has been attributed to the absorption of the blood-coloring matter of the medium. The colonies usually remain isolated. Successive cultures are obtained by transference to new media containing sterile blood. This blood need not necessarily be derived from human beings, that of animals also sufficing. Transfers made at intervals of more than four days are not likely to succeed. In order to obtain cultures from material containing other bacteria, Kruse recommends the use of agar plates covered with rabbit's or pigeon's blood, upon which the bacteria-containing material is spread with a platinum brush. In this way single colonies can be obtained for further cultivation. Huber found that the blood coloring-matter is as useful as the entire blood for obtaining cultures. According to Pfeiffer, growth does not take place in the absence of oxygen, and only very slightly in the depth of stabs. Kamen, however, has grown the organism in hydrogen as well as in the attenuated atmosphere obtained by the use of alkaline pyrogallic-acid solutions

(Buchner's method). He finds that the bacilli are sometimes more resistant to Gram's stain, and also that cultivated anaerobically they live for three weeks, whereas they die within from ten to fourteen days when grown exposed to the air.

Pathogenicity.—Influenza bacilli have been found in the expectoration and in the secretions of the nose and bronchi in large numbers in cases of influenza occurring in Europe and in this country. In typical acute cases they are present in almost pure or even in pure cultures in the purulent greenish-yellow bronchial secretions. Kamen, in his examination of one hundred cases, succeeded in isolating the influenza bacilli in all. In order to insure success the sputum is collected with great care, the mouth first having been rinsed out with sterile water, and the sputum received into sterile dishes. Mucoid or purulent particles are selected for making the cultures. The morning sputum brought up from the bronchi is to be preferred. Stained preparations are best made with dilute Ziehl's carbol-fuchsin. The bacilli lie in nests or masses. At first the bacilli may be free, but in the later stages they are found in the protoplasm of leucocytes, and as the disease progresses the number of bacilli in the sputum usually diminishes. It is stated that as the number decreases the staining becomes less sharp and involution forms appear. The bacilli are present not only in the secretions, but also in the substance of the lung where they can set up both in adults and in children pneumonic processes (Pfeiffer, Finkler, Weichselbaum, Ribbert, Meunier). The nasal secretion may contain them in enormous numbers. The inflammation of the lungs may extend to the pleura, which may also become involved, while more distant points, such as the pericardium (Högersted), the endocardium (Flexner and Austin), the central nervous system (Pfuhl and Walter), the middle ear (Bulling), may become the seats of inflammation, due to the localization of this bacillus. Meunier was able in cases of infantile bronchopneumonia to cultivate the organism directly from the aspirated juice of the lung, and from the circulating blood obtained from veins. Haedke describes an instance of meningitis and epidural abscess secondary to influenza otitis in which influenza bacilli were cultivated from the pus in the ear and that in the meninges. Slawyk has reported an instance of general infection of the body with special localizations in the joints (hand, foot, and knee) and meninges. Chiari has cultivated the bacilli from the spleen in a case of influenza pneumonia. Influenza infections are usually acute or subacute processes, and very commonly, especially in the air passages and lungs, the specific bacilli are associated with pneumococci, streptococci, and staphylococci. Pfeiffer has pointed out that the bacilli may, however, be present during many

months in association with more chronic diseases of the lung, at times producing acute exacerbations. Finkler has observed this tendency especially in consumptives. Neisser has reported a case of aneurism of the aorta in which there was pneumonia. The influenza bacillus was obtained from the focus, there had been no symptoms of influenza during life. Notwithstanding the statement of Canon it is now generally agreed that the bacilli in uncomplicated cases of influenza do not appear regularly in the blood current. Animals are only slightly susceptible to inoculation with cultures of the organism. According to Pfeiffer, monkeys show a response to intraperitoneal injections or applications of cultures to the nose by a febrile reaction, lasting some days; while injections into the subcutaneous tissues produce abscesses. An actual increase of the organisms in the body cannot be said to have been demonstrated in these inoculations, and it is probable that the effects may be entirely toxic. Delius and Kolle have injected considerable quantities of cultures into mice, rabbits, and guinea-pigs. Intraperitoneal inoculations, if the quantities are large, may cause death in from twelve to forty-eight hours. According to these authors, under such circumstances there is an increase of the bacilli in the peritoneal cavity. Much larger amounts are required when the inoculations are subcutaneous or intravenous; in these instances no increase of the organisms is supposed to take place, death being attributed to a toxæmia. One-half cubic centimetre of the fluid obtained by lumbar puncture by Slawyk killed a guinea-pig in thirty minutes after intravenous injection.

Attempts to produce immunity in animals have been followed by entirely negative results. Cantani, Jr., studied the effects upon rabbits of the bacilli and the toxin attached to their cell protoplasm. Injections were made directly into the brain. Relatively small doses proved fatal, death taking place in from eighteen to thirty-six hours, the symptoms being fever, reaching its height in twelve hours, and followed by a remission in which the temperature became subnormal. In sublethal doses the animal recovered, but a chronic meningitis was produced. In fatal cases the organisms were found in the brain surrounded by leucocytes. Increase had apparently taken place, the bacteria being disseminated through the lymphatics. The bacilli were also found in the spinal cord, but never in the blood, peritoneal fluid, or organs generally. Repeated passages of the organism through a series of animals by intracranial inoculation produces a marked increase in the virulence. Intracranial injections of influenza bacilli previously killed by heat (57° C. for one and one-half hours) cause death of the animals, the symptoms being the same as those produced by the living cultures.

Bacillus Pseudo-Influenzæ.—Obtained by R. Pfeiffer in three cases of bronchopneumonia following diphtheria. These cases occurred during the prevalence of an influenza epidemic. Also found by H. Kossel in otitis media in sucklings, and by Hartmann, Pilecke, and Kruse in a supposed case of influenza in an adult. The bacilli are non-motile, small, and do not stain by Gram's method. The individuals are slightly larger than the true influenza bacillus. Growth occurs upon culture media prepared as for the latter, but a difference is noted in that there is a tendency to the production of somewhat thicker pseudo-threads. The colonies are similar to those of B. influenzæ. The organisms were evidently pathogenic for man. Animal experiments are not recorded (Kruse).

Diphtheria Bacillus Group.

Small, slender, often unsymmetrical, non-motile bacilli. Behavior towards Gram's stain variable. Asporogenous. In specimens from pathological processes and young cultures a tendency to stain irregularly is exhibited. Some forms show pronounced pathogenicity (B. diphtheriæ, B. pseudotuberculosis of Welch and Kutscher). Others are widely distributed saprophytes (B. pseudo-diphtheriæ, B. xerosis). In some of these forms branching of the rods has been observed. Growth takes place slowly at room temperature, better at that of the thermostat. Gelatin is not liquefied, and the individual colonies are small, the general growth not being abundant.

Bacillus Diphtheriæ. *Synonym.*—Klebs-Loeffler bacillus of diphtheria.

First observed and described by Klebs in sections of diphtheritic membrane. Isolated by Loeffler, who regarded it as the probable cause of true diphtheria. His observations were soon confirmed by Roux and Yersin in France, Kolisko and Paltauf in Austria, and Welch and Abbott in the United States, as well as by a number of German bacteriologists.

Morphology and Cultural Properties.—Non-motile bacilli of small size, which in young cultures show marked irregularities of form, such as swollen, wedge, and flask-shaped rods which are sometimes irregularly crooked or curved. With some anilin stains (methylene blue) a marked irregularity of chromatic distribution is brought out, so that individual members may suggest appearances resembling short chains of cocci. The individuals vary from 0.5–1 to 1–6 μ in length. A characteristic arrangement of the bacilli into palisade-like figures both in cultures and in tissues was observed by Kruse, who describes it in the following way: "The dividing bacilli have a deli-

cate spindle form. At the moment of division each member exhibits a flattened edge at one end, the two being usually in contact. This union does not last long, for one of the members, probably through the influence of the pressure of growth, turns itself so that it at first forms a right angle with its fellow, but soon is arranged parallel with it. Such continued divisions and rearrangements bring about a peculiar palisade formation." Cover-slip preparations show not alone considerable differences in form, but also great variations in length, these alterations in part depending upon the medium upon which they are grown (Abbott). The diphtheria bacillus stains by Gram's and Weigert's methods. No spores are observed. The highly refractory bodies contained within their substance are feebly resistant, and are killed in half an hour by a temperature of 60° C.

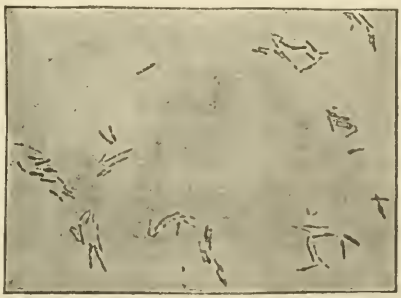

Fig. 23.—Bacillus Diphtheriæ (after Park).

Dried specimens in not too thin a layer live for months. Cultures have been made from a bit of membrane preserved for five months in a dry state. Incorporated with dust and kept moist, the bacilli were still cultivatable at the end of eight weeks, but in the dried state they no longer grew at the end of this period (Ritter). In cultures great variation is found, some specimens living for months, others dying at the end of a few days. Media which permit of much acid formation (glycerin-agar) are not adapted for preserving its vitality. Low temperatures have little influence upon it. The bacilli grow very feebly at temperatures of 20° C. and lower. At 24° C. growth is more abundant, while at the temperature of the thermostat it is at its best. The most characteristic growths are obtained upon blood serum, the medium best adapted for this purpose being that suggested by Loeffler (see page 586). Glycerin-agar, though serving the purpose, is far inferior to it. Ordinary agar is so poor a medium as not to be adapted for the isolation of the organism from mixtures of bacteria such as usually occur in the human throat. Growth is usually completed at the end of forty-eight hours in the thermostat.

The colonies on blood serum are white, opaque, and tolerably firm. Those upon glycerin-agar are more translucent, gray, and less sticky. Both upon blood serum and agar they are thoroughly characteristic. They show a peculiar granulation, thickest in the centre, and least marked in the periphery, the edge showing an irregular crenation. The deep colonies are small, dark, granulated, with irregular edges. The growth in stabs and upon stroke cultures depends partly upon the amount of material transplanted, and partly upon the medium. If small amounts of material are transplanted individual colonies are prone to develop. With a larger quantity growth takes place along the entire stroke of the needle and spreads for from one-half to one millimetre beyond it. In bouillon in some instances a uniform turbidity is obtained, reaching its height in one to two days, while at other times granules develop which fall to the bottom, or adhere to the side of the tube, leaving the fluid quite clear. Potato usually gives an invisible growth. Cover slips, however, show a great increase of the organisms, many of which exhibit marked irregularity in form and staining-properties. Milk is a good medium; it is not coagulated. In litmus-milk a moderate amount of acid is created. Indol is produced, but somewhat irregularly. The organism can be grown, although with some difficulty, in asparagin and salt solutions (C. Fraenkel, Uschinsky).

For the purpose of isolating the diphtheria bacillus from the air passages in cases of diphtheria, Loeffler's blood serum is to be preferred. Agar media permit too free a growth of pyogenic organisms with which the diphtheria bacillus is usually associated in these situations.

Klein, Kanthack, and Babes first described slight branching of this organism, an observation which was neglected until rediscovered by C. Fraenkel. Such organisms, in which a single or at most two branches appear, have since been obtained by many bacteriologists both from the throats of human beings and from cultures.* A medium composed of egg albumen is especially well adapted to the production of the branching forms.

Pathogenicity.—The diphtheria bacillus is the admitted common cause of pseudomembranous inflammations of the throat and nose. A small percentage of instances are due to other microorganisms, particularly the pyogenic cocci. In the true diphtheritic inflammations of these parts, although there is not infrequently an admixture

* F. N. Meyerhof has studied this condition and concludes that branching is not due to abnormal development, but indicates a relationship with the streptothrices. He does not, however, go as far as Lehmann and Neumann, who would class B. diphtheriæ with the hyphomycetes.

of other organisms, pyogenic cocci, capsulated bacilli, pseudodiphtheria bacilli, B. diphtheriæ predominates, and tends to exist alone in the freshly forming deeper parts of the membrane. The organisms can be demonstrated in cover-glass preparations directly from the false membrane, but even when this method fails they may commonly be obtained in cultures on Loeffler's blood serum and glycerin-agar. In the trachea the false membrane often contains the diphtheria bacilli in pure cultures. Sections of the diseased parts show the diphtheria bacilli to be, as a rule, the most deeply situated microorganisms, occurring in the form of zooglœa-like masses. The diphtheria bacillus develops for the most part locally, so that the general symptoms in the disease are due chiefly to the local production of the poison which is absorbed and transported by the circulating blood. It is certain, however, that the internal organs may become the seat of invasion with bacilli. The organisms have been cultivated from the blood of the heart after death (Flexner, Bovar). The bronchopneumonic foci in human beings associated with diphtheria contain this organism pure or admixed with pyogenic cocci (Kutscher, Wright, Flexner, Kanthack and Stevens, and others). Flexner and Anderson injected suspensions of bacilli into the trachea of rabbits and produced a fatal pneumonia. The bacilli are also found in the adjacent lymph glands (Bullock and Schmorl), while small numbers of them have been obtained from the blood and the various organs of the body by cultural methods. The foci of bacilli in distant organs may in part account for the localized lesions found there, but the morbid changes are chiefly due to the development in the throat, the organic lesions, such as cell degenerations and necroses, degeneration of nerves, being referable to the action of the diffusible poison produced. Rare examples of pesudomembranous inflammations due to the diphtheria bacillus are found in the conjunctivæ and in the skin (E. Fraenkel, Brunner, Abel, and others). Pseudomembranous otitis media is sometimes, though more rarely, associated with the diphtheria bacillus, although the secondary suppuration of the middle ear is commonly caused by this organism. The pseudomembranous inflammations of the throat associated with exanthematous diseases, such as scarlet fever and measles, are more often due to streptococci than to the diphtheria bacillus. These cases are characterized for the most part by a more favorable course than those of true diphtheria. It has been proposed to speak of these pyogenic pseudomembranous inflammations as pseudodiphtheritic. Diphtheria bacilli are pathogenic for guinea-pigs, rabbits, chickens, pigeons, small birds, and cats, and in a less degree for dogs, goats, cattle, and horses. Mice and rats are refractory. In all the susceptible animals the chief

effects are referable to the poison produced by the organism. Excepting the local lesion, which in guinea-pigs bears some resemblance to the false membrane in human beings, all of the morbid effects, both clinical and pathological, of the diphtheria bacillus can be produced by injections of the toxin (Welch and Flexner). The pathological pictures in guinea-pigs poisoned with the diphtheria bacillus or its toxin are characteristic. The animal dies within periods varying from twenty-four hours to several days. In some instances death is delayed for several weeks (Roux and Yersin). The subcutaneous inoculation of living cultures in rapidly fatal cases produces in guinea-pigs a local hemorrhagic œdema, as well as the transudation of clear fluid in the pleural, pericardial, and peritoneal cavities. A small, opaque, whitish area appears at the site of inoculation, especially if solid cultures be used, consisting of necrotic tissue, fibrin, and leucocytes, while diphtheria bacilli occur distributed in a manner suggestive of the histological picture presented by the pseudomembrane in human beings. The adrenal and thyroid glands are always swollen and hemorrhagic. The microorganisms are demonstrable in the local lesion, and in small numbers in the internal organs (Wright). Abbott and Ghriskey have produced small lymphoid nodules in the omentum by injecting cultures into the testes of male guinea-pigs. Flexner has observed fibrinopurulent peritonitis from direct injections into the peritoneal cavity. In animals that live for longer periods, the chief symptom is the loss of weight. In such animals, besides the ordinary lesions described, paralyses due to degeneration in the peripheral nerves (Sidney Martin, and others) have been observed. Direct inoculation of the diphtheria bacillus into the trachea, conjunctiva, and vagina of animals produces pseudomembranous inflammations of these mucous membranes. Subdural inoculation is rapidly fatal, secondary metastases being found in the lungs setting up lobular pneumonia (Flexner).

Mode of Infection.—In the great majority of cases of diphtheria in human beings there is conclusive evidence of either direct or indirect transmission of the infectious material. Not all instances, however, can be traced to a certain origin. The study by bacteriological methods of healthy individuals who have been brought into contact with patients suffering with diphtheria has shown that virulent organisms are carried by them in their throats, upon other parts of their persons, and in nurses especially in the hair over the ears, the most favorable place for contact with the infected fingers (Wright). Not all persons who carry the virulent microorganisms in their throats are necessarily attacked by diphtheria; on the other hand, many mild examples of angina have been shown to be associated with virulent

diphtheria bacilli, and the suspicion has been aroused that these trifling instances of disease are not infrequently the means of dissemination of the poison, which in more susceptible individuals produces typical and often severe diphtheria. Virulent diphtheria bacilli have been found in the throats of healthy persons not known to have been associated with those suffering from diphtheria, but more frequently the organism isolated under these circumstances has been the pseudo-diphtheria bacillus (see below). There are instances of laboratory infection, from the accidental aspiration of cultures of the diphtheria bacillus, presenting the clinical picture of pharyngeal diphtheria.

Not infrequently it happens that after the disappearance of all clinical symptoms diphtheria bacilli can still be cultivated from the throat, the length of time which they persist varying from a few days to several months. Fibiger has observed an instance in which the bacilli were present for nine months; while in another case Hewlett and Nolan isolated the organisms six months after the recovery of the patient. On the other hand, according to Simonin and Benoit, the average time for their persistence in ordinary cases is thirty-four days, while in masked cases (D. larvata) the average is from sixty-three to eighty-three days. Kreling found in a case which had been treated with antitoxin that the bacilli were still cultivatable from the nineteenth to the thirty-first day.

Poison Production.—The studies of Roux and Yersin, Brieger and C. Fraenkel, and others have shown us that this organism in cultures produces a poison which is separable from the bacilli by filtration. The quantity of poison and the rapidity of its formation depend upon the original virulence of the bacilli employed and the composition of the culture media. In the presence of sugar very little poison is generated until this substance is entirely broken up. In sugar-free media, on the other hand, poison production is rapid (Th. Smith). The exact chemical nature of the poison is unknown. For the present it may be regarded as a proteid, and therefore entitled to the name of toxalbumin, although the recent studies of Brieger and Cohn, and Brieger and Boer tend to controvert this idea. By special methods of preparation they have obtained a poison which gives only a very faint biuret reaction. By precipitation with lime salts the poison can be obtained in a dry, impure form. A similar poison has been demonstrated by Wassermann and Proskauer in the bacteria-free fluids (transudates, urine, etc.), and organs of infected animals.

Immunization.—Recovery from infection with the diphtheria bacillus in human beings and animals is associated with increased resistance. The inoculation of animals with modified cultures, through which their virulence has been diminished, permits of a higher degree

of immunization being achieved. Various methods have been employed, such as the heating of cultures to 45° C. (Ferran), and the employment of chemicals, such as trichloride of iodine, carbolic acid, etc.; and the treatment of infected animals by means of chemicals, among which may be mentioned trichloride of iodine and hydrogen peroxide (Behring), has been tried with greater or less success. At the present time large animals, such as goats and horses, are immunized by injections of repeated and increasing doses of filtered cultures containing the poison. Animals treated in this way yield, according to the law formulated by Behring, a serum which is both protective and curative. This serum, obtained from the horse, preserved by the addition of small quantities of chemicals (carbolic acid, tricresol) and carefully standardized, is now obtainable in commerce under the name of diphtheria antitoxin. This antitoxic serum has been employed with great benefit in the treatment of various forms of diphtheria in human beings. As a prophylactic somewhat smaller doses are injected into persons exposed to diphtheria infection. Certain secondary effects, such as erythema and swelling of the joints, referred to the effects of the horse's serum, are believed to be in no way connected with the peculiar antitoxin itself.

As regards the advisability of making protective inoculations of an antitoxic serum in the case of persons who have been exposed to diphtheria, there is considerable difference of opinion. Variot has spoken against it, whereas Löhr, Hilbert, and Tavel are strongly in favor of such a procedure.

Differential Diagnosis.—Not all pseudomembranous inflammations of the throat and other parts are caused by the diphtheria bacillus. Organisms belonging to the same general group as B. diphtheriæ are now known occasionally to occur in the human throat and upon the conjunctivæ in health, and to be perhaps somewhat increased in numbers in pathological states. The determination that the diphtheria bacillus is the cause of a given pseudomembranous inflammation can as a rule be readily made on account of the striking morphological and pathogenic properties of the organism. The morphological criteria are found in the bizarre forms and the irregular staining of the diphtheria bacillus, and in its resistance to Gram's stain and the positive reaction to Neisser's specific stain (see p. 680). The next important characteristic is the appearance presented more especially upon blood serum and upon glycerin-agar, while the final proof is to be brought by the inoculation of guinea-pigs, which react with perfectly typical symptoms. Minute quantities of highly virulent cultures cause the death of these animals in from eighteen to forty-eight hours. The inoculated animals, six hours after the injections, become

quiet and show a tendency to seek a dark and obscure corner of the cage, while their hair loses its soft appearance and smooth arrangement, and looks coarse and ruffled. The animals refuse to eat and become gradually weaker, although they are usually able to maintain the standing position until within a very short time before death. Very small doses of virulent cultures, and somewhat larger doses of less active ones, produce at the point of inoculation a marked localized swelling, which in rare instances ulcerates, the ulcer later undergoing cicatrization. These animals may die at a somewhat later period, five to seven days, or not until after the lapse of several weeks; more rarely they recover altogether. The various other membranes of the xerosis group of bacteria, including the pseudodiphtheria bacillus, are devoid of virulence, and the susceptible guinea-pig does not react to them, even when administered in large doses.

Examination of Cultures for Diagnostic Purposes.—Many cities in the United States and Europe, through the local health organizations, offer to make bacteriological examinations for diphtheria without cost, and to report within twenty-four hours upon all cultures sent in. The carrying out of this arrangement is facilitated by distributing at convenient points small boxes, each containing a tube of Loeffler's solidified blood serum and a sterilized swab with which to spread the suspected material upon the surface of the medium. The tubes collected from these various sources are put into the thermostat, where they are allowed to remain over night. At the end of twelve to fourteen hours in the incubator, if the diphtheria bacilli are present, they have already grown abundantly, and have outstripped the pyogenic organisms with which they are usually associated. Cover-slip preparations made from such cultures, stained with one of the ordinary anilin dyes or by Gram's method, and examined microscopically, suffice for purposes of immediate diagnosis. The presence of a considerable number of bacilli having the morphological peculiarities of the diphtheria bacillus, while not absolutely pathognomonic for the existence of diphtheria, may for all practical purposes be regarded as sufficient proof. It is customary to report positively upon such specimens without waiting for the examination of pure cultures and the making of animal experiments. The fact that the pseudodiphtheria bacillus is usually present in small numbers in the throat is considered sufficient reason for eliminating this organism from consideration in interpreting the bacteriological findings. The large statistics collected in New York, Philadelphia, and numerous large cities abroad have given unequivocal support to these diagnoses, and the sanitary importance of the early recognition of cases of diphtheria justifies such errors as must, of course, occasionally occur.

Bacillus Pseudodiphthericus; Bacillus Xerosis.—Bacilli resembling in some respects B. diphtheriæ, but distinguished from it by certain cultural and staining properties as well as by the absence of pathogenicity. The pseudodiphtheria bacilli were first described by Loeffler and by von Hofmann-Wellenhof, who regarded them as being non-virulent forms of the Loeffler bacillus. Later investigations have shown that this bacillus is very widely distributed, and occurs in the normal secretions of the mouth and throat. A similar bacillus, showing, however, certain cultural differences from the pseudodiphtheria bacillus, has been repeatedly found in the conjunctival sac in health and disease. To this organism the name of xerosis bacillus was given by Kuschbert and Neisser, who found it present in large numbers in the condition known as xerosis conjunctivæ—an observation which has since been confirmed by many other investigators. Roux and Yersin showed that the Loeffler bacillus possessed great variations in virulence, and that, in fact, it was sometimes innocuous; and they were of the opinion that pseudodiphtheria bacilli represented non-virulent forms of the Loeffler bacillus. Considerable controversy has raged between bacteriologists with regard to the identity of these microorganisms. Loeffler, von Hofmann, Roux and Yersin, Koch, Dunbar, Abbott and Schanz believe the two groups to be merely modified forms of the Loeffler bacillus. On the other hand, Hueppe, Fraenkel, Escherich, Spronck, and Trumpp hold that they are entirely different from the Loeffler bacillus, and have nothing to do with the production of diphtheria, so that they should be regarded as distinct species. Later investigations, including the comparative study of Bergey, are in support of the establishment of this distinction. Bergey concludes that the pseudodiphtheria bacillus and the xerosis bacillus are not only distinct from B. diphtheriæ, but also from each other. He considers it preferable to designate as the pseudodiphtheria bacillus an organism whose colonies form a thick, creamy white layer on agar-agar and on blood serum. The distinction from the Loeffler bacillus depends upon the character of the growth, but more especially upon the observation that the pseudodiphtheria bacillus is almost never capable of producing any lesions whatever in animals, and when exceptionally it exercises any morbid effects these are of a mild and local character. Furthermore, it fails to give the characteristic staining by the procedure introduced by Neisser, which is as follows: A solution is made by dissolving 1 gm. of powdered methylene blue (Grubler) in 20 c.c. of ninety-per-cent. alcohol, and then adding 950 c.c. of distilled water and 50 c.c. of acetic acid. For a second stain, 2 gm. of vesuvin are dissolved in a litre of boiling water, and the solution is then filtered. The cover slips are stained in the first solu-

tion for from one to three seconds, washed in water, and then stained from three to five seconds in the second solution, again washed in water and examined. Diphtheria bacilli grown on Loeffler's blood serum at temperatures from 34° to 35° C. for ten to twenty hours show a double staining, in which the chromatin granules appear as blue dots on a faintly yellow ground. The pseudodiphtheria bacilli treated in the same way do not give the double-stained picture. B. xerosis differs from the true diphtheria bacillus in that the growth upon agar and blood serum consists of minute dry pearly-white colonies, showing no tendency to coalesce except when sown very thickly, when they grow as a dull, thin, pearly-white layer. Furthermore, it displays a lack of virulence and gives no reaction to the double staining-method of Neisser. From the pseudodiphtheria bacillus B. xerosis is distinguished chiefly by the extent of growth and the size of the colonies. The distinctions pointed out by Zarniko—according to whom the pseudodiphtheria bacillus is incapable of changing the reaction of bouillon, while the true diphtheria bacillus produces acid under these circumstances—has been shown to be unreliable, since it has been proved that different organisms show considerable variations in this respect—variations depending in part upon the composition (presence of sugar) of the culture medium. Organisms having the morphology of the diphtheria bacillus, but devoid of virulence, probably belonging to the group of pseudodiphtheria and xerosis bacilli, have been described in human beings in association with a number of diseases, such as Egyptian dysentery (Kruse and Pasquale). They have also been demonstrated upon the skin, in the crusts of variola pustules, and in impetigo, in sputum, in a case of mild angina, and apex pneumonia (Kruse). Ohlmacher has found them in a case of pneumonia in which the organism was admixed with other bacteria, and they have been isolated by Babes in gangrene of the lung, by Howard in ulcerative endocarditis, by Ravenel in membranous rhinitis, by Weichselbaum in endocarditis (B. endocarditis griseus), by Harris in ascitic fluid, by Bergey in pus and pyuria, by Wilde in ozæna, and by Schültz and Ehret in a considerable number of cases of pulmonary tuberculosis. Both the pseudodiphtheria and xerosis bacilli show occasional branchings (Kanthack, Prochaska, Schanz).

Bacillus Pseudotuberculosis Murium.—Obtained by Welch from a spontaneous epidemic of pseudotuberculosis in mice in Baltimore, and afterwards by Kutscher from cheesy nodules in the lungs and pleuræ of a mouse found dead. Similar bacilli were obtained by Preisz and Guinard from cases of pseudotuberculosis in the sheep.

Morphology and Cultural Properties.—Non-motile rods, about the size of diphtheria bacilli, which show similar irregularities of form and staining. In bacilli from the animal body as well as from old cultures, and cultures grown under high temperatures, a marked tendency to simple branching has been observed by Dorothy Reed. The colonies as well as the growths upon blood serum and agar-agar are very similar to those of B. diphtheriæ. On potato there is an invisible growth.

Pathogenicity.—Large laboratory animals, such as the rabbit and guinea-pig, are resistant. Mice are susceptible, but only rarely die as the result of subcutaneous inoculation. Positive results may be obtained by injecting small amounts of the cultures into the peritoneal and pleural cavities. The bacilli develop locally and in the viscera, producing minute translucent nodules, and upon the serous surfaces in addition a relatively thick grayish pseudo-membrane. Injections into the pleural cavity usually cause pleuritis and pericarditis and lobular consolidation of the lung, with nodular formations. The occurrence of these nodules has led to the employment of the term pseudotuberculosis, but, as D. Reed has shown, the nodules are not composed of proliferated cells as in tuberculosis, and in fact contain very few cells at all, but consist of colonies of bacilli arranged somewhat regularly and showing at the periphery simple branching. These colonies bear resemblance to the *Drusen* of actinomycosis.

Differential Diagnosis.—The organism is differentiated from the true diphtheria bacillus by its pathogenicity for mice, its absence of pathogenic effects upon larger laboratory animals, and its failure to react in the characteristic manner when stained by Neisser's method. From the pseudodiphtheria bacilli it is readily distinguished by its pathogenicity for and effects upon mice.

Group of Glanders Bacillus.

This group of organisms contains the well-defined B. mallei, and several other bacilli which cause pseudotuberculosis. These have been brought together by Kruse, and can be conveniently treated under this heading, although they do not possess the characters of a definite group. Most of them are small bacilli, occurring singly or in chains, and are asporogenous. They grow upon the ordinary culture media, and are pathogenic.

Bacillus mallei. *Synonyms.*—Rotzbacillus; Bacille de la morve. Isolated by Loeffler, Schütz, O. Israel, Bouchard, Capitan, Charrin, Weichselbaum, Kranzfeld, and Kitt.

Morphology and Cultural Properties.—Slender, sometimes slightly bent, small bacilli, varying in width from 0.25 to 0.4 μ and in length

from 1.5 to 3 μ. Non-motile, although often exhibiting very active Brownian movement. In old cultures the bacilli undergo involution, and break up into irregular, small, almost coccus-like elements. They are stained with some difficulty with the ordinary anilin dyes, and are decolorized by Gram. The rods often show irregularities in staining, being made up of deeply stained granules and unstained bodies. These latter have been mistaken for spores. The bacilli showing these unstained portions are not more resistant than those in which the staining is more regular. Kruse has found that in thin layers the bacilli upon drying quickly die. Loeffler, however, has reported one instance in which he obtained living bacteria from material which had been kept dried for many months. In distilled water the bacilli are injured after some days. According to Bonome they are killed at 70° C. in six hours, while at from 90° to 100° C. three minutes suffice. The bacilli grow upon ordinary media, although best upon glycerin-agar and upon potato. Blood serum and ordinary agar are less well adapted to their growth. On glycerin-agar, at the end of twenty-four to forty-eight hours, the colonies are white and transparent, the fully developed ones reaching several millimetres in diameter. Upon potato there is an elevated reddish-brown growth several millimetres in thickness. The organisms grow upon gelatin at temperatures from 20° to 25° C. but slowly. After some weeks the gelatin is slightly liquefied upon the surface. The cultures vary in their vitality upon these various media, living longest upon gelatin. Acid media are well adapted to the growth of the organism. Indol is produced, although slowly. Lewandowski has also noted the presence of phenol in cultures.

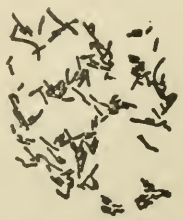

Fig. 24.—Bacillus Mallei. × 1,000. (Fränkel and Pfeiffer.)

Pathogenicity.—Glanders appears as a natural infection almost exclusively in horses and donkeys. Human beings who have to do with these animal are sometimes infected. Cases of laboratory infection have been observed. The lesions in animals appear primarily in association with the mucous membrane of the nose, less frequently with the skin and internal organs, such as the lungs. According to Cornil and Babes, skin infection can occur without previous lesions of the part; Nocard has succeeded in causing infection by feeding contaminated material to horses and donkeys. The lesions occurred in the submaxillary lymph glands, the larynx, and the nasal mucous membrane. At the autopsy, caseous nodules were found also in the lungs. In human beings lesions of the nasal mucous membrane are far less common than those of the skin and muscles. The superficial

manifestations take the form of extensive bullous or pustular exanthems, phlegmons, carbuncles, or abscesses, which appear in the skin and muscles. The involvement of the lymph glands (farcy), so common in animals, is far less marked in human beings. The chronic form of glanders (the *Wurm* of the Germans), observed in animals, is only rarely found in human beings. Duval, Gasne, and Guillemot succeeded in cultivating bacilli during life from the blood in a case of human glanders. Glanders bacilli are pathogenic for a variety of animals. The most susceptible is the guinea-pig, which usually succumbs after subcutaneous inoculation in from two to four weeks. Ulceration occurs at the seat of inoculation; the organisms extend to the neighboring lymph glands, nodules appear in the spleen and lungs, and caseation of the testes and vulva, together with suppuration of the joints and the nasal passages usually take place. The results of intraperitoneal inoculation are more rapid, death usually occurring in from eight to ten days. For the purpose of differential diagnosis, suspected material is suspended in bouillon or sterilized water, and injected into the peritoneal cavity of male guinea-pigs. At the end of the second day the testes are already perceptibly swollen, while by about the sixth or seventh day the scrotum is also involved, the swelling has greatly increased, and ulceration of the skin may take place. The lesions found at autopsy consist of nodules in the spleen, omentum, and liver, with extensive caseous infiltration of the tunica albuginea testis. This diagnostic method, which was introduced by Straus, is commonly employed in suspected cases. It is generally considered conclusive, although, according to Nocard and Kutscher, an orchitis may be produced by other organisms (see below). Again, in rare instances the localization in the testes may be wanting. Among other animals, which are susceptible to inoculations with cultures of or material containing glanders bacilli, are the cat, which succumbs in from eight to twenty days, the dog and the hedgehog, in from five to fourteen days, the field-mouse, in from two to eight days, the wood-mouse, in from two to three weeks, and the marmot. The last animal, however, is sometimes immune. It has been observed that beasts of prey in menageries, such as lions and tigers, which have been fed with infected meat, succumb to the lesions of glanders. Rabbits are less susceptible, although they usually respond to inoculation. Sheep, house-mice, swine, hens, and pigeons are much more resistant, whereas cattle are immune. The bacilli are most abundant in young glanders nodules, although, as Kitt has shown, caseous and partly calcified nodules may still contain them. For the purpose of diagnosis mallein, which consists of the soluble products of the growth of the bacilli, has been extensively

employed. Injections in animals suffering from glanders produce at the end of from four to ten hours a febrile reaction, while the tissues at the site of inoculation become œdematous. MacFadyen has observed that the blood serum of a horse suffering from glanders produces agglutination of the bacillus mallei. The specific nodule of glanders has been found by Baumgarten to be the result of the proliferation of the fixed cells, connective-tissue cells, the cells of blood-vessels, and epithelial elements, which later undergo degeneration, break down, and attract polymorphonuclear leucocytes. According to J. H. Wright, in the guinea-pig the primary visceral lesions show necroses of epithelial cells rather than proliferation.

Bacillus Orchiticus.—Isolated by Kutscher from the nasal secretions of an animal from which the glanders bacilli had also been obtained. The bacilli appear as non-motile rods, resembling glanders bacilli, but staining by Gram. Heating to 55° C. kills them in five minutes. They are said to grow upon all ordinary culture media excepting milk. On gelatin liquefaction is quite rapid, the colonies resembling those of the cholera vibrio. In guinea-pigs intraperitoneal inoculation produces swelling of the testes, appearing at the end of forty-eight hours, and death in from four to five days. Nodules are found in the omentum and testes, but rarely elsewhere. Subcutaneous inoculations in small doses kill at the end of from one to two days. Mice also are susceptible even to small doses injected subcutaneously. This organism is differentiated from the glanders bacillus by its pathogenicity for ordinary mice, and its staining by Gram.

Nocard obtained a bacillus from animals, which showed an ulcerative lymphangitis of the skin, in some respects resembling the lesions of glanders. It possesses virulence very similar to that of B. orchiticus. It differs from this organism, however, in growing not at all in gelatin at the room temperature, and but slightly at the temperature of the thermostat. It is said also to grow in milk, although poorly. It stains by Gram's method, and is demonstrable in the pus from the lesions of the skin.

Bacillus Pseudotuberculosis (Pfeiffer). *Synonym.*—Streptobacillus pseudotuberculosis rodentium. Obtained by A. Pfeiffer from the organs of a horse supposed to have suffered from glanders. It is doubtless identical with the organism described by Mallassez, Viginal, Chantemesse, Nocard, Eberth, Charrin, Roger, and others. The bacilli appear as plump rods, averaging 0.4 μ in thickness, and about 1.2 μ in length, often united into chains. Not infrequently the bacilli are very short, and resemble cocci. They stain in the ordinary anilin dyes, but are bleached by Gram's method. They are non-motile and asporogenous. They grow in ordinary media, and do not liquefy

gelatin. According to Pfeiffer, milk is not altered. All rodents are more or less susceptible to subcutaneous and to intravenous inoculation as well as to feeding. Rats, cats, dogs, hedgehogs, horses, goats, and field-mice are less susceptible. According to the mode of inoculation death takes place at the end of a few days or weeks, the lesions consisting of nodules resembling tubercles. The histological picture is characterized by accumulations of lymphoid, epithelioid, and giant cells, but the exudative character of the process predominates over the proliferative. The bacilli are present in the nodules, very frequently lying within the cells. A differential diagnosis from the glanders bacillus and B. tuberculosis is easily established by the cultural and pathogenic properties, and from the latter also by differences in staining-qualities.

Bacillus Pseudotuberculosis Liquefaciens.—Obtained by Cazal and Vaillard from caseous nodules in the peritoneum of a man. Larger nodules were present in the pancreas and liver. The centre of these nodules contained large masses of bacilli which were easily cultivated. They were short, motile, and often appeared in chains. The bacilli are asporogenous; they do not stain by Gram; they grow readily upon all ordinary culture media and liquefy gelatin. Mice succumb to subcutaneous inoculation with a general infection of the body, but without showing specific lesions. Guinea-pigs are refractory. Rabbits die from intravenous inoculation in from one to two months, showing at autopsy caseous nodules in the subcutaneous tissue and in the thorax. Subcutaneous inoculation is also successful, but large quantities are required.

Bacillus Ulceris Cancrosi. *Synonym.*—Bacillus of soft chancre. Although this organism is incapable of cultivation, and its properties are, therefore, very imperfectly understood, it is for the present classed by Kruse in this group. It probably was first observed by Ducrey in 1889, who obtained it by successive inoculations of the secretion of a soft chancre from individual to individual. In this way he finally isolated from the secretion of his experimental inoculations a single organism—a small bacillus occurring within the pus cells, and not staining by Gram's method. This observation has been confirmed by Krefting. More recently Unna found in five excised soft chancres a bacillus which appears in long chains, does not stain by Gram's method, occurs particularly in the superficial infiltrated layers of the ulcer, but lies outside of cells. According to Unna this streptobacillus is the cause of the lesion. It is probable, although not certain, that the two organisms are distinct. The demonstration of the Unna bacillus in the tissues is, according to Kruse, relatively simple. His method consists in staining the sec-

tions in Loeffler's methylene blue, dehydrating quickly with alcohol or anilin oil, clearing in xylol, and mounting in balsam.

Group of the Hemorrhagic Septicæmia Bacilli.

These appear as small bacilli, usually observed as isolated short rods, asporogenous, not staining by Gram's method. They are facultative anaërobics, are usually readily cultivated upon ordinary culture media, and do not liquefy gelatin. They stain by any of the ordinary anilin dyes, and often show deeply stained poles, the central portion of the rod remaining uncolored. On account of this property the organisms are sometimes referred to as the *figure-of-eight* bacilli. In their pathogenic action they produce bacteræmia. They are concerned with the production of destructive diseases among the lower animals. Similar bacilli have in a few instances been found in human infections.

FIG. 25.—Bacillus Choleræ Gallinarum. × 950. (Baumgarten.)

BACILLUS CHOLERÆ GALLINARUM. *Synonyms.*—Bacillus of chicken cholera or chicken plague; Bacterium avicidum; Bacillus of rabbit septicæmia; Bacillus cuniculicida, etc. First obtained by Perroncito, then by Toussaint and Pasteur, R. Koch, Babes, and others. It was studied more especially by Pasteur, who found it to be the cause of chicken cholera.

Morphology and Cultural Properties.—The bacilli usually appear as non-motile, short rods, but vary somewhat in size. They average from 0.4 to 0.6 μ in width, and are 1 μ in length. In stained preparations diplococcus-like forms are often found. Longer forms, however, are also observed in the same cultures. The organism is sensitive to heating and drying. Temperatures of 45° and 46° C. destroy the virulence of cultures in thirty minutes. Under ordinary conditions, however, the virulence is retained for months, and is not affected even by the admixture of putrefactive bacteria. In plate cultures the colonies in the depth appear as round or irregularly spherical points, while those upon the surface spread slightly, often showing a central nucleus. The stab cultures show a growth along the lines of the stab which may be uniform, or may show separate colonies. The growth upon potato at the temperature of the thermostat is translucent, waxy, grayish-white. Bouillon is slightly clouded, and indol and phenol are produced. The action upon milk is variable; sometimes alkalies are produced, while, at other times, according to C. Fraenkel and Kruse, acids appear and coagulation ensues. Lehmann and Neumann found that both glucose and lactose are converted into acid, although gas is not formed.

Pathogenicity.—These bacilli are typical septicæmic organisms, and are pathogenic, even in small doses, when introduced subcutaneously or by feeding, for pigeons, chickens, geese, ducks, pheasants, small birds, and birds of prey, as well as rabbits and mice. Guinea-pigs, sheep, and horses are less susceptible, and usually react with local suppuration. Guinea-pigs occasionally die as a result of a general invasion of the body by the organisms. Dogs and cats, as well as man, resist the ingestion of infected meat. In the bodies of animals which have succumbed great numbers of the organisms are found in the blood-vessels. The local lesions in pigeons and chickens consist of necrosis of tissue with hemorrhagic infiltration, the intestine showing the lesions of a hemorrhagic enteritis. In these animals, as well as in rabbits, pneumonia is also said to occur. Hemorrhages may be found in the serous membranes. The bacilli are transmitted from mother to fœtus, and in the case of birds also to the egg (Marchiafava and Celli). Pasteur was able to produce the drowsiness, so prominent a symptom in chicken cholera, by injecting filtered cultures. The organism is very widely disseminated in nature. Koch found it in stagnant water, while Gamaleïa claims that slightly virulent forms are constant in the intestinal contents of healthy pigeons. By successive inoculations in rabbits and pigeons the virulence is increased.

With this organism Pasteur made the first observations on protective inoculation. He had observed that cultures many months old, which did not kill inoculated animals, would protect them from another infection with virulent material. By artificially reducing the virulence of cultures preventive inoculations have been carried out on a large scale with successful results.

Bacillus Suisepticus. *Synonyms.*—Bacillus of swine plague; Schweineseuche Bacillus.

Morphology and Cultural Properties.—The bacilli average 0.8 to 1.4 μ in length, and often present the appearance of slightly oval bodies, more like cocci than bacilli. Sometimes they appear as rods of considerable length. They are non-motile, asporogenous, and do not stain by Gram. They grow on the ordinary culture media, with the exception of potato. Gelatin is not liquefied. The gelatin and agar growths are grayish, translucent, not extending far from the point of inoculation, and resemble the growth of the bacillus of chicken cholera. Bouillon cultures are sometimes diffusely cloudy, but more frequently the growth is in the form of a whitish, rather viscid sediment, or appears as little specks in a clear fluid. According to Welch, when planted on potato, there may be a feeble, invisible growth for one or two generations, probably due to the transference

of a little of the nutritive medium to the potato with the organisms. The vitality in cultures is variable, and usually short. In cover-glass preparations from the fresh juices and tissues of animals the bacilli present a typical polar staining, unless the forms are very short, when the staining is uniform.

Pathogenicity.—The organism is the cause of the disease designated in the reports of the United States Bureau of Animal Industry, issued since 1886, as swine plague. The swine-plague bacillus occurs in swine, and in these animals is frequently associated with the hog-cholera bacillus. The bacilli are most commonly found, either alone or mixed with hog-cholera bacilli, in the hepatized lung and in the exudates on serous membranes. Sometimes they are confined to these localities, but at other times they are found also in larger or smaller number in the blood, spleen, kidneys, liver, lymphatic glands, and intestine. The infection, therefore, with these organisms may be either local or general. According to Welch and Clement no definite lesions are referable to their presence, excepting in the lungs, where they may cause pneumonia, and in the serous cavities, where they produce inflammations. Inoculation of swine with pure cultures of the swine-plague bacillus, as carried out by Welch and Clement, are described as follows: "The bacilli manifest marked variations in virulence, some varieties possessing slight or no virulence when tested on swine, while others are markedly virulent. The duration of life in fatal cases varies from sixteen hours to from eight to ten days, rarely more. The fatal results may take place from subcutaneous inoculation, but this is exceptional. Intravenous inoculation of large doses is generally fatal; of smaller doses occasionally fatal, often not. By this method of inoculation there may be produced fibrinous pleurisy and pericarditis, both with and without pneumonia. Direct inoculation into serous cavities is sometimes fatal, and produces more or less extensive fibrinous inflammations. Inoculation directly into the lungs, or into the trachea, sets up a characteristic pneumonia. In addition there may be produced pleurisy and pericarditis. Feeding of cultures, as well as of the bodies of animals dead of swine plague, produces no effect. In some fatal cases a hemorrhagic enteritis is produced, but no diphtheritis, as is the case with the hog-cholera organism. Rabbits are also susceptible, and two main types of virulence can be distinguished in these animals. One kind kills rabbits by subcutaneous inoculation in from sixteen to thirty hours. The bacilli have undergone enormous multiplication in the blood and organs. In the other the rabbits die in from two to six days, occasionally after a longer period, with extensive seropurulent infiltration around the site of inoculation, often with peritonitis, and frequently

in these cases with few bacteria in the blood and organs, but an immense number in the inflammatory exudates. Mice are also susceptible." V. Moore has found swine-plague bacilli in the air passages of a number of healthy animals. They were pathogenic. Smith and Moore produced artificial immunity in rabbits and guinea-pigs. They used sterilized cultures in bouillon and agar, sterilized blood of infected rabbits, and the blood serum of previously immunized rabbits.

Bacillus Diphtheriæ Columbarum (Loeffler). Described by Loeffler as the cause of diphtheria in pigeons, an observation confirmed by Babes and Puscariu.

Morphology and Cultural Properties.—Non-motile rods, somewhat longer and thinner than the organism of rabbit septicæmia. They are asporogenous and do not stain by Gram. The growth upon culture media is similar to that of the typhoid bacillus, except that on potato a grayish membrane develops. The organism is said not to produce indol.

Pathogenicity.—The organism occurs naturally in epidemics of diphtheria in pigeons. Mice, young pigeons, small birds, and rabbits are susceptible. Chickens, guinea-pigs, and rats are more resistant. Dogs are non-susceptible. Mice succumb at the end of from four to nine days, and show splenic tumor and numerous necroses in the liver, in the midst of which masses of bacilli are found. Bacilli also appear in the blood and organs generally. Subcutaneous inoculation in pigeons produces a local swelling with ulceration, from which the animals usually recover. If the mucous membrane of the mouth is scarified and the bacilli are fed to the animals in water, a diphtheritic membrane appears that may return after desquamation, and death may take place at the end of one to three weeks. Bacilli are present in the membrane as well as in the internal organs. The intestine shows hemorrhagic enteritis. Rabbits when inoculated in the eye are attacked by a pseudomembranous conjunctivitis. The cultures gradually lose virulence through artificial cultivation.

Voges[30] has made a comparative study of the group of hemorrhagic septicæmic bacilli. Certain differences, as for example in the growths of the bacilli of German and American swine plague, are shown to be dependent upon the artificial conditions of culture. The variations in pathogenicity also are not considered as indicative of specificity. Very slight attention has hitherto been paid to racial peculiarities of animals and the dose and virulence of the bacteria. Along with the improvement in the breeds of swine a diminution in resistance to infection takes place. Voges was able to increase the virulence of cultures so that one one-hundred-millionth part of a

drop, approximately a single bacillus, killed guinea-pigs in from five to eight hours. Even motility is subject to fluctuation. There are no bacterial criteria through which differentiation of several of the hemorrhagic septicæmias, notably chicken cholera and swine plague, can be made. The cultures are little poisonous at first; it is only after the bacterial cells to which the poison is attached disintegrate that they become toxic. Normal blood serum of guinea-pigs and some other animals possesses bactericidal and antitoxic properties to the bacillus of swine plague and allied varieties. Active immunity in animals is bactericidal rather than antitoxic and is soon lost. Kitt and Mayr have observed the interaction of the blood sera of immunized animals for swine plague and chicken cholera.

Bacilli Producing Hemorrhagic Infections in Human Beings.

Hemorrhagic infections in human beings have various origins. Many of them are associated with the pyogenic organisms, others with the presence of the capsulated bacilli, while in some obscure instances investigators have isolated microorganisms resembling, if not identical with, the hemorrhagic septicæmia bacilli of animals. I shall follow Kruse's classification in giving briefly some of the examples of this form of infection, including, for the present, under this heading the bacillus of bubonic plague.

Bacillus Hæmorrhagicus Nephritidis.—Obtained by Vassale from a case of hemorrhagic nephritis in a puerperal woman, in which it was associated with a streptococcus. In morphology it resembles the bacillus of chicken cholera, is only slightly pathogenic for rabbits but highly so for guinea-pigs, the latter animals developing hemorrhagic septicæmia.

Bacillus Hæmorrhagicus Septicus.—Obtained by Babes from three cases of hemorrhagic septicæmia associated with stomatitis, angina, purpura, hæmaturia, and other symptoms. The organisms were found in hemorrhagic foci in the lungs, in the mesenteric glands, and in the spleen. They are capsulated, and grow very poorly upon ordinary media. They are pathogenic for mice, giving rise to hemorrhagic septicæmia in these animals.

Bacillus Hæmorrhagicus.—Cultivated by Kolb from the cadavers of three persons who had suffered from hemorrhages into the skin and mucous membranes. The bacilli are also capsulated and grow somewhat more freely than the preceding. Mice are susceptible, dying in from two to three days from a hemorrhagic septicæmia. Rabbits succumb to intraperitoneal injections, but guinea-pigs are more resistant.

Bacillus Hæmorrhagicus Velenosus.—Obtained by Tizzoni and Giovannini from a case of purpura hæmorrhagica that developed secondary to impetigo contagiosa. The bacilli were present in the impetigo pustules associated with the staphylococcus aureus, and also in the liver and blood. The organism is pathogenic for dogs, rabbits, and guinea-pigs, but not for pigeons and mice when introduced subcutaneously. The bacilli develop only locally, but all the symptoms of the original disease, including hemorrhages, are produced.

Bacillus Exanthematicus.—Obtained by Babes and Oprescu from a case of febrile hemorrhagic infection of the petechial type. The bacilli are numerous in the capillaries in the liver, and in the kidneys. They are motile, tolerably plump, and sometimes appear in a figure-of-eight form. Some of the organisms stain by Gram's method. They grow fairly abundantly in agar. On potato there is a grayish and later a brownish layer. Bouillon becomes cloudy with the production of a membrane. The organism is pathogenic for mice, rabbits, guinea-pigs, and pigeons.

Bacillus Gingivitidis.—Obtained by Babes from an epidemic of scurvy in Jassy, being isolated from the ulcers on the mucous membrane of the mouth. It was associated with a streptococcus and the bacillus of chicken cholera. The excised mucous membrane of the mouth showed beneath the fibrinous membrane a thick felt-work of bacilli. The organisms appeared as long, slender bacilli, varying from 0.3 to 3 μ, or even more, in length. Rabbits, guinea-pigs, and dogs succumbed to subcutaneous injections. The bacilli were cultivated from the mucous membrane of the gums and from the hemorrhages in the rabbit. When they are injected together with the streptococcus fatal hemorrhagic infections are produced. Similar bacilli were found by Babes in the deposit on the teeth in healthy persons.

Bacillus Pestis. *Synonym.*—Bacillus pestis bubonicæ. Discovered by Yersin in 1894 during the prevalence of an epidemic in Hong-Kong.

Morphology and Cultural Properties.—Motile, very short rods, which in length are so variable that some of the members appear almost spherical, others distinctly rod-like. They occur singly or in chains. They are about of the thickness of B. coli, but are usually shorter than that organism. Flagella surround the rods (peritricha). Spores are not formed. Staining is easily accomplished by means of ordinary dyes, and is often most pronounced at the poles. Gram's stain gives negative results. A capsule is demonstrable especially in pathological exudates. Motility is slight, and easily demonstrable only in young colonies. Cultures may be obtained both upon agar-

agar and gelatin. The medium should be slightly alkaline and not too moist. Agar slants present a white thin, moist, translucent growth, that is not characteristic. Pfeiffer prefers gelatin to agar, especially when the number of bacilli present in the exudate or blood is small. Colonies upon agar-agar are grayish-white in color, and usually present an iridescent translucency. Under the low powers of the microscope they are moist, almost round, and very irregular, with indented edges. The young colonies present a glass-wool-like appearance. As they become older they develop greater opacity. Gelatin stab cultures at the end of three to five days appear as a white thread, which on the surface spreads slightly into a thin membrane. There is no liquefaction. Growth takes place on potato in the form of a grayish-white, dry, thin cuticle. At low temperatures this medium is not well adapted to growth, the optimal points lying between 30° and 32° C. From 20° to 25° C. the growth is much slower, about twice as much time being required for a given degree of development. At much lower temperatures, as for example between 4° and 7° C., growth may still take place, although it proceeds very slowly. The organism resists a freezing temperature for a long time, although according to Forster such cultures develop more slowly when afterwards subjected to the optimum. Loeffler's blood serum is well adapted to growth, which is especially well marked in the condensation water of this medium, the chain-like forms being particularly well developed. Bouillon is not clouded, but gives a characteristic growth which has been compared with stalactites. Crumbly flakes of growth also sink to the bottom or adhere to the walls of the tube. Drying, according to Gaffky, quickly kills the organism. The rapidity with which they are destroyed depends upon the thickness of the layer, the surrounding temperature, and the medium. In warm climates they are destroyed more quickly than in cooler regions. According to Forster, bouillon cultures are killed partly by the drying process, but partly also by the concentration of the solution. In eight-per-cent. salt solution the bacilli succumb very quickly. Flügge dried pest bacilli with fine dust, which upon being atomized was found to be entirely sterile. Dried upon silk threads and kept in the dark at the room temperature, the organisms are still alive at the end of fifty-six days (Loeffler). Dried upon woollen fibre they are still alive at the end of forty-five days (Forster).

Pathogenicity.—This bacillus is the admitted cause of the bubonic plague. It has been found in the several countries in which the plague has prevailed since 1894: in China, India, and certain European countries into which the disease has been introduced (Portugal). According to the studies carried out by Aoyama in China,

and by the German and Austrian commissions in India, as well as those of Lowson in China and India, the portal of entry into the human body may be the external skin, the respiratory mucous membrane, the mouth, the conjunctivæ, and perhaps also the intestine. Barker and Flint saw cases in India in which the parotid became infected, it is supposed, through Stenson's duct. Localization is less common in the skin or the mucous membrane, but when it takes place in these situations a pest carbuncle or furuncle makes its appearance. In a certain percentage of cases the primary lesions can be made out, especially on the extremities. Instances of this kind were noted by the writer in Hong-Kong. The primary localization is more frequently in the lymph glands in immediate relation with the point of inoculation, giving rise to the pest bubo. The inguinal glands in men are most frequently attacked, for the reason that the natives, among whom the infection chiefly develops, are accustomed to go barefoot, or cover the feet and legs only imperfectly, and are therefore inoculated in the lower extremities. In native women the lower extremities are usually covered so that the point of inoculation is generally in the hands or arms, and the buboes are most common in the axillary region. Involvement of the cervical lymphatic glands results from tonsillar infection; the bronchial and mesenteric glands become involved secondarily after infection through the lungs or the intestine respectively. Primary infection of the lungs may give rise to pest pneumonia. The microorganism invades the blood and metastases occur, manifesting themselves as secondary buboes in various lymphatic glands, secondary pneumonia, meningitis, as well as localized foci in the liver, kidneys, and other organs. According to Wilm in twenty to thirty-five per cent. of the cases of bubonic plague occurring in Hong-Kong primary buboes were absent, the disease presenting the clinical picture of septicæmia. At autopsy all the lymphatic glands and especially the mesenteric glands were found to be much swollen. The intestinal mucosa was hemorrhagic, epithelial defects were demonstrable, but no carbuncle could be demonstrated in the mucous membrane. Wilm looks upon these cases as primary intestinal forms of infection. Pfeiffer endeavored to determine whether the bacilli can be absorbed from the uninjured skin and mucous membranes. He found that the rubbing of cultures into the shaved skin of animals proved positive. The inoculation of small quantities of cultures upon the uninjured mucous membrane of the nose, or the introduction of fluid cultures into the mouths of animals gave rise to pulmonary infections. The atomization of cultures in fluids and their aspiration also caused lung infection. The German commissioners from their work in Bombay deny the existence of pri-

mary intestinal infection, and interpret somewhat differently the appearances described by Wilm as characteristic for this variety of infection. They point out that the primary pest bubo is distinguished by its richness in bacteria, and by the hemorrhagic and œdematous infiltration of the surrounding tissues. Secondary buboes, on the other hand, contain few bacilli, while the periglandular structures are not infected. According to Pfeiffer and Stricker, the appearances described by Wilm belong to this second category. Experimental animals react, the rat being especially susceptible. These animals can be infected by feeding upon cultures or upon the flesh of other animals which have succumbed to the plague. The fæces and urine of these animals contain the bacilli in large numbers. The various races, white, mottled, gray, and black, are equally susceptible. Mice are more resistant, and as a rule do not succumb to feeding. Guinea-pigs react to subcutaneous and other inoculations, but are very difficult to infect by feeding. With regard to swine, opinions are divided. The German commissioners are of the opinion that they are insusceptible. Wilm, on the other hand, succeeded in infecting in Hong-Kong two pigs, by feeding them with the spleen from a fatal case. In the case of one of these animals, which died at the end of four weeks, the spleen gave cultures which were fatal to rats. Cultures are kept virulent by passage from animal to animal, whereas artificial cultivation causes the bacilli gradually to lose their activity. Maassen preserved a culture for two years in a sealed glass tube protected from the light; upon inoculation this was proved to have retained its original virulence, whereas a sub-culture from the same source cultivated during two years had in the same time entirely lost its activity. According to Loeffler, cultivation upon blood serum and preservation at low temperatures maintain the virulence.

It is admitted that rats play an important part in the dissemination of the pest bacilli. There is reason to believe that prior to an epidemic among human beings, the rats suffer extensively from the plague. There are numerous instances in which large numbers of these animals were found dead before cases of plague had begun to develop in man. The rôle of mice is not established, although it is stated that in Formosa they have played as important a part as rats in other places. According to Simond, whose statements as yet lack confirmation, fleas are capable of carrying pest from infected to healthy rats, and perhaps also to human beings. Flügge holds that mild cases of the pest are very important in the dissemination of the disease. Gotschlich has reported three cases of pest pneumonia in which the sputum contained virulent pest bacilli for many weeks after recovery. In the first case they were demonstrable on the seventy-

sixth day, which was forty-eight days after the apyrexia was established. In the second case they were still demonstrable on the thirty-fifth day of the disease, and in the third case on the thirty-third day after the cessation of the fever.

Poison Production.—That the pest bacilli possess toxic properties could be assumed from the general symptomatology of the disease. To these are to be ascribed the prostration and depression, as well as the hemorrhages in the serous membranes and internal organs. An interesting confirmation of this view has been adduced by Stricker, who reports that in three fœtuses, whereas the secondary effects of the disease, such as hemorrhages and parenchymatous degeneration, had occurred, the bacilli nevertheless could not be demonstrated. The poison, as in the case of the Bacillus typhosus and the Bacillus of Asiatic cholera, adheres to the bacterial cell. The filtrates in general are inactive. Old bouillon cultures are, however, poisonous. Lustig and Galeotti have extracted a nucleo-proteid from the plague bacilli which possesses toxic and immunizing properties.

Immunization.—Haffkine's protective inoculations made in India are carried out by injecting from 1 to 3 c.c. of bouillon cultures, which have been killed by exposure to 70° C., and to which 0.5 per cent. carbolic acid has been added. These injections produce reactions, after which a protective effect is manifested. The dose for children of the so-called "serum" is less. Pfeiffer recommends the employment of agar cultures forty-five days old. These are first killed and the carbolic acid is then added; after a period of twenty hours, during which the chemical is permitted to act, the injection may be made. Lustig has produced active immunity in animals, including monkeys, by injections of solutions of his nucleo-proteid, and he has also tested its effects upon man. He found no ill effects from its use while its protective properties seem established. It has the advantage over Haffkine's "serum," of being of more definite composition. Passive immunity (for example, by the injection of a serum obtained from immunized animals) has proved certainly useful only among the lower animals. This immunity which requires large quantities of serum for its production disappears in a few days. The method may, however, prove serviceable in certain cases, since the active immunity resulting from the injection of sterilized cultures does not appear until about the eighth day. The combination will also probably serve to eliminate the dangers sometimes encountered from the injections of cultures alone. Lustig and Yersin in India claim to have obtained excellent results in the treatment of plague in man.

The *serum diagnosis* of pest offers difficulties. In the first place it is more difficult than with the typhoid bacillus to obtain cultures

in which the microorganisms are uniformly distributed, since they show a marked tendency to grow in conglomerate masses. A second difficulty is met in the fact that agglutination does not occur in all cases; moreover, the reaction, in opposition to what occurs in typhoid fever, appears not during the height of the disease but during convalescence. It has been shown by Pfeiffer that normal human serum has no action in dilutions of 1:1; and he believes that when agglutination occurs, the conclusion may be drawn that the individual has recovered from the plague. On the other hand, the absence of agglutination does not indicate the non-existence of the disease. It is desirable that this test be made on a larger scale in order that the limits of its usefulness may be ascertained.

Group of Mouse Septicæmia Bacilli.

These are small bacilli, asporogenous, stainable by Gram's method. They grow moderately upon ordinary culture media, and are highly pathogenic for animals.

Bacillus Murisepticus. *Synonyms.*—Bacillus of mouse septicæmia (Koch); Bacterium murisepticum (Migula). It was first obtained by R. Koch by inoculating mice with putrefactive fluids, and is a widely disseminated organism.

Morphology and Cultural Properties.—In cultures the organisms appear as delicate rods, varying from 2 to 4 μ in length, and 0.4 to 0.6 μ in width, usually straight, but sometimes slightly curved, and united into threads. When obtained from the blood and the organs of animals they are usually shorter and more slender, being about 1 μ in length and 0.1 to 0.2 μ in width. They are non-motile, and stain by the ordinary dyes, and also according to Gram. They are facultative anaërobics. Gelatin plate cultures at the end of from three to four days show very delicate indentations, in which the colonies appear as extremely fine, veil-like membranes. On magnification the colonies can be seen only if the light is very much reduced. In gelatin stabs the growth is delicate, and a special feature is the presence of very fine, hair-like projections going off at right angles to the stab —the so-called fir-tree appearance. These branches usually unite, and a cloud-like translucent growth appears in the medium, the surface gradually becoming depressed and cupped. Gelatin is very slowly liquefied. The growth upon agar-agar, both in plate cultures and in stabs, is less characteristic. The isolated colonies are more opaque and coarser, and the growth along the line of the stab is less abundant than upon gelatin, the side branches, which are so characteristic in the latter medium, being entirely absent. Bouillon is

clouded; there is a slight sediment, but no membrane. Milk is rendered amphoteric or slightly alkaline; it is not coagulated. There is no perceptible growth upon potato. According to Lehmann and Neumann neither indol nor H_2S is formed.

Pathogenicity.—With one exception, a spontaneous epidemic in Greifswald among mice, this organism has been found only in experimental infections. Domestic mice, but not field-mice, are susceptible. They usually die in from two to three days, numerous organisms being found in the fluids and organs. Pigeons are also susceptible, dying in from two and a half to three and a half days (Th. Smith). Rabbits and guinea-pigs are resistant, whereas swine are at first made sick, but usually recover.

Bacillus Rhusiopathiæ Suis (Kitt). *Synonyms.*—Bacillus of swine erysipelas, Schweinerotlauf Bacillus; B. rouget du porc; Bacterium erysipelatos suis (Migula).

Morphology and Cultural Properties.—This organism is very closely related to the bacillus of mouse septicæmia. Its microscopical appearances and staining-reactions are identical. In the gelatin stab culture great similarity is also observed, the branches, however, sometimes being a little thicker and more brush-like. Inoculations made directly from blood into gelatin, according to Lorenz, in the first cultures may lack the usual branches. The chief point of difference, first pointed out by Loeffler, is in the appearance of the colonies upon gelatin plates. These, which consist of small, quite distinct membranes sending out branches, bear a good deal of resemblance to bone corpuscles. In its growth, H_2S is produced in large quantities, and indol in small amount. The organism was discovered by Pasteur, and afterwards by Loeffler, Schütz, Lydtin, and Schottelius, who have contributed the most important studies. Drying quickly kills the bacilli. They remain alive a long time, however, in putrefying fluids. At a temperature of 52° C. they are killed usually in fifteen minutes, although they sometimes resist up to a temperature of 70° C. According to Kitt and Petri, in larger masses of flesh they are difficult to kill by cooking, curing, salting, and smoking.

Pathogenicity.—The bacillus of swine erysipelas is the cause of destructive plagues, especially among the younger animals of the best breeds of swine. The older as well as the younger animals of the ordinary races are more or less immune. Animals dead of the disease often show a diffuse or mottled reddening of the skin, subcutaneous œdema, injection of the pharynx, stomach, and mucous membrane of the intestine, swelling of the mesenteric glands and spleen, parenchymatous changes in the kidney, associated sometimes with

hemorrhage, and a mottled congestion of the lungs. The organism is harmless for human beings; hence the ingestion of flesh obtained from diseased animals is not injurious. It is pathogenic for mice, both white and gray, white rats, and pigeons. Mice may be infected by ingestion of the organism. Rabbits, however, die only after direct inoculation. In the animals dead of the infection, bacilli are present in the blood, often being contained in large numbers in the protoplasm of the leucocytes. Mice which eventually succumb to this infection, usually in the last hours or days of life occupy a corner of the cage, in which they sit motionless, with the eyelids glued together by secretion, and the head drawn in as though they were asleep. They most commonly die in this position. Field-mice and wild mice, guinea-pigs, cattle, horses, donkeys, dogs, cats, hens, geese, and ducks are refractory. Sheep are said to be slightly susceptible. In susceptible swine infection may be produced by rubbing cultures into the skin.

Immunity.—Animals which have recovered from the disease are immune against reinfection. An artificial immunity was obtained by Pasteur and Thuillier by injecting subcutaneously attenuated cultures obtained by continued cultivation in artificial culture media, or by first transferring the organism to less resistant animals, as, for example, the rabbit. Small animals may also be immunized in the same way. Emmerich and Mastbaum have utilized the juices obtained from protected animals for the purpose of immunizing other animals against infection, as well as for therapeutic purposes.

Anthrax Bacillus Group.

This group consists of large, easily cultivated, sporogenous bacilli, often united into pseudo-threads. Gram's and Weigert's stains are positive. The pathogenicity is variable in different species or varieties of the members.

Bacillus Anthracis. *Synonyms.*—Bactéridie du charbon; Milzbrand Bacillus; Bacterium anthracis (Migula).

Morphology and Cultural Properties.—Large, slender, cylindrical, non-motile rods, varying from 1 to 1.25 μ in thickness and from 4 to 7 μ in length. In cultures one often sees pseudo-threads, in which under suitable staining are seen individuals, of which the length is scarcely twice the breadth. In the bodies of susceptible animals the rods appear isolated, or united into short chains. The extremities of the bacilli vary; the free ends are generally rounded, whereas those in contact are flat, the bamboo-like excavations being artefacts due to imperfect technique. In the animal body the rods are surrounded

with a stainable capsule, which by special staining methods can also be demonstrated in the artificial cultures. The bacilli stain readily with all anilin dyes and with hæmatoxylin. Gram's method is especially suitable for staining them in sections. The spores are ellipsoidal, the length being one and a half times or even twice the breadth. Their first appearance is indicated by a highly refractive granule in the substance of the rod. This becomes larger and larger, while at the same time the mother cell loses some of its contents. The threads in which the spores have wholly developed resemble a string of beads. In some instances the formation of spores is very irregular and appears only here and there in the course of the threads. Asporogenous varieties can be artificially produced. The disintegration of the mother cell liberates the spore, which is capable of germinating both in fresh culture media and in the animal body. In the course of the germination the high refraction is gradually lost. The spore swells up and extrudes the young rod through the ruptured end of the capsule. The germination of spores can be observed directly under the microscope in the hanging drop at a temperature of 37° C., the whole process being completed in a few hours. By special methods (see page 608) double staining of the spore and of the vegetative substance can be obtained. In unfavorable media remarkably irregular, as well as clumped and spherical, etc., involution forms are met with. These degenerated bacilli show irregularities of staining. The anthrax bacillus grows readily upon all ordinary culture media, but poorly upon Uschinsky's fluid. The organism is well adapted to saprophytic existence.

Fig. 26.—Bacillus Anthracis, from a Culture, showing Spore Formation. × 1,000. (Klein.)

The most characteristic growth is obtained in gelatin. At a temperature of 24° C., at the end of twenty-four hours, small point-like colonies are formed, which under the low power have a dark gray or greenish appearance, and show an irregular outline. As growth continues, the periphery grows more and more irregular because of small projecting and elevated masses, until the interior appears striated

and so convoluted as to justify the term "Medusa-like." Coincidently the gelatin begins to liquefy. The finished colonies average from 2 to 4 mm. in diameter. The gelatin stab is also characteristic. At first there develops a delicate white line; this begins at the top, and gradually grows into the surrounding medium in the form of brush-like or hair-like prolongations. Liquefaction begins after two days, proceeding from the surface to the depth, until finally complete liquefaction of the medium is accomplished, the culture sinking to the bottom of the fluid. The growth upon agar plates is similar to that upon gelatin, but is somewhat less compact and characteristic. The agar stab shows growth along the puncture, and also upon the surface of the medium. There is no liquefaction. The growth upon blood serum is abundant, the medium being slightly liquefied. On potato a grayish-white dry membrane appears, but fails to extend over the entire surface. Bouillon cultures are usually clear, the growth settling to the bottom. A typical membrane is not formed, although sometimes fragments of growth float on the surface of the fluid. Milk is coagulated through the action of the lab ferment, and is afterwards peptonized. The reaction is usually not changed. According to Fermi and Maumes, the bacillus also produces a diastatic ferment. Indol is said not to be formed, although the writer has frequently obtained it in old peptone-water cultures. Growth is not obtained as

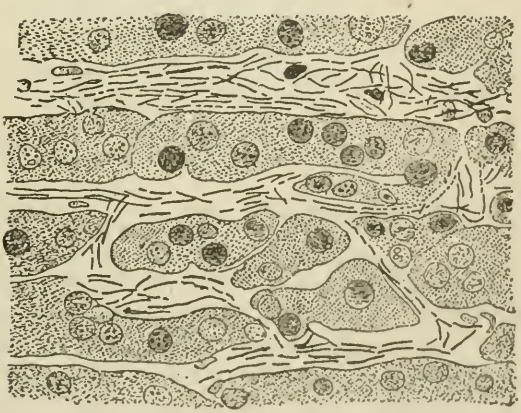

Fig. 27.—Bacillus Anthracis in the Liver of a Mouse. × 700. (Flügge.)

a rule under 12 to 14° C., although the organism may be acclimated to these temperatures. At the other extreme, growth is still obtained at 42 to 43° C., but at this temperature spores are only rarely formed. The addition of antiseptics to culture media also prevents the formation of these bodies. At the body temperature growth is rapid and

spore formation is abundant. Spores, however, are not demonstrable in the bodies of infected animals immediately after death, though later under certain conditions sporulation is possible. Upon agar and other favorable media spores are already present at the end of twenty-four hours, and sporulation is complete at the end of a few days. For demonstrating spores in cultures potato is an especially favorable medium. The bacillus is a facultative anaërobic, although the cultures grow much more abundantly in the presence of air. The growth that takes place under anaërobic conditions is, according to Sanfelice, associated with the loss of the power to produce the pep-

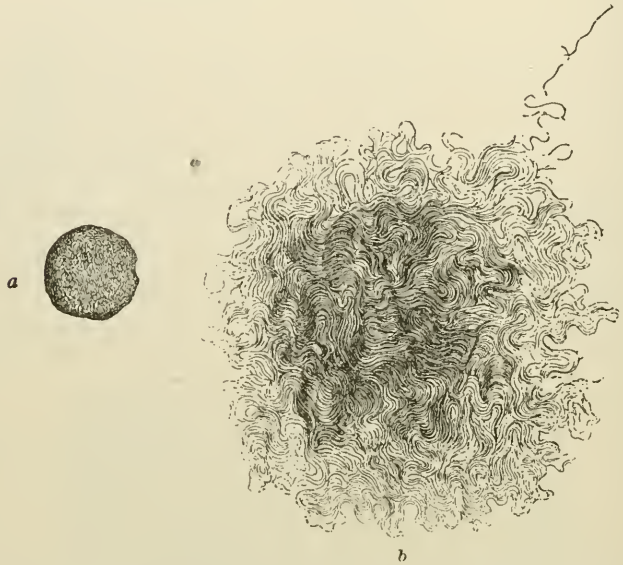

FIG. 28.—Colonies of Bacillus Anthracis on Gelatin Plates. *a*, At the end of twenty-four hours; *b*, at the end of forty-eight hours. × 80. (Flügge.)

tonizing ferment. For sporulation the free access of oxygen is necessary. This fact has led to the belief that the absence of spores inside of the animal body is attributable to the small amount of oxygen there available. At depths of 1.5 metres in buried animals and at a temperature of 15° C. sporulation takes place but slightly, whereas at a depth of 2 metres, where the temperature is still further reduced, it is entirely wanting (Kitasato). Spores live for months and years; the vegetative forms are much less resistant. Bacilli free from spores, dried in the presence of a fluid rich in albumin, have been found living at the end of sixty days, whereas dried bouillon cultures, according to Momont, will succumb in twenty-one days. Dried spores kept at the ordinary temperature retain their vitality for years. The

direct rays of the sun destroy them in four hours, and exposure for many weeks to diffuse daylight has the same effect (Kruse).

Pathogenicity.—Anthrax as a natural disease occurs in domesticated and wild animals and in man. It has the distinction of being the first infectious disease the bacterial nature of which was definitely proven. In the blood of cattle dead of anthrax, Pollender in 1849, and Brauell and Rayer a little later, demonstrated the bacilli. Experimental evidence of their relation to the disease was brought first by Brauell, and afterwards by Davaine. R. Koch first cultivated the bacilli and demonstrated their sporulation. In human beings, anthrax occurs chiefly as a skin infection through wounds and possibly the bites of insects. More rarely infection takes place through the intestinal and respiratory tracts. The maximal fatality is observed in infections of the intestine or of the respiratory tract. In the milder cases the microorganism remains localized, whereas the fatal forms are associated with a general infection of the body. The most common type of dermal anthrax infection is characterized by the appearance of the carbuncle or malignant pustule, to which in the severer forms œdema is superadded. In the early stages the bacilli are demonstrable in cover slips from the infected area, but later on they undergo involutions so as to be unrecognizable as such, although they can be still brought out by cultural and animal experiments. The bacilli are most numerous in the upper layers of the corium; they are also found in the papillæ, and more rarely in the depths of the tissue. Intestinal anthrax is far less common in human beings than in animals, perhaps for the reason that men are less exposed to the ingestion of spores. Cases have been observed in persons—butchers, brush makers, and others—whose daily work had brought them into constant contact with materials contaminated with spores. The lesions in the intestines consist of hemorrhagic, tumor-like elevations of the mucous membrane, containing large numbers of microorganisms. From this situation entering the general circulation, they may become localized in the peritoneum, giving rise to peritonitis, or upon the heart valves, producing vegetative endocarditis (Flexner, Blumer, and Young). Pulmonary anthrax has been described by Eppinger and Paltauf in rag-pickers, and in persons employed to sort contaminated rags in paper factories. The disease runs a course varying from two to seven days. The pathologico-anatomical picture consists in swelling of the tonsils, the formation of necrotic patches in the air passages, bronchitis, hepatization and œdema of the lungs, serous pleurisies, swelling of the bronchial glands, œdema of the mediastinum, acute swelling of the spleen, etc. Greenfield has described, under the name of wool-sorters' disease,

similar inhalation anthrax. According to Schottmüller the bacilli appear in the sputum and in the blood in this form of infection. Bratanich describes an outbreak of meat-poisoning in Shönau, Bohemia, due to a mixed infection with anthrax and trichinæ. Chiari obtained from the spleen in a fatal case of this outbreak bacilli having the cultural properties of the anthrax bacillus, but failing to form spores, and non-virulent. Rabbits inoculated with this germ were protected against fatal doses of virulent anthrax bacilli. Spontaneous anthrax is relatively common in sheep and cattle, but occurs more rarely in horses and swine. V. Rátz reported an epidemic among swine occurring in 1895 in Hungary. It is extremely uncommon in smaller animals. Sheep and cattle suffer more particularly from the intestinal form of infection, and experimentally they develop the disease from the ingestion of small quantities of spores, although the vegetative bacilli are quickly killed by the gastric secretion. The mortality among the animals attacked averages from seventy to eighty per cent. Bacilli are abundantly present in the blood, and the organs show lesions of an acute septicæmia. The spores develop on the surface of the intestine, the bacilli penetrating the intestinal mucosa and follicles and entering the circulation. Sheep are also susceptible to skin infection, with the exception of the Algerian race, which, according to Chauveau, is resistant to anthrax. Cattle, on the other hand, react only slightly to skin inoculations unless large quantities of the material be used. Mice, both white and gray, are extremely susceptible, dying from inoculation with minimal quantities of the bacilli, and also succumbing to the weakened virus. Guinea-pigs are next in order in respect to susceptibility, while rabbits are slightly more resistant. Infection can be obtained by inoculating into the tissues or into the blood, while feeding with spores is frequently unsuccessful. Experimental pulmonary infections may be positive, and according to Buchner are more likely to succeed with the spores than with the rods. Rats are far less susceptible, the white rat being somewhat less resistant than gray and black rats. Cats, hens, pigeons, and dogs are distinctly more resistant. In the small susceptible animals subcutaneous inoculations produce a gelatinous œdema, in which the bacilli are present, although in much smaller numbers than in the blood and internal organs, where they are found packed inside the capillaries. Indeed, so thick are they in these vessels that properly stained specimens sometimes give the impression of a vascular injection. The organs generally show lesions of an acute general infection, one of the chief characteristics being the swelling and softening of the spleen. In pregnant females the bacilli may pass from the mother to the fœtus. In human beings this condition

has been observed by Rostowzew. In three cases examined by him the organisms were more abundant in the liver of the fœtuses than in the circulation of the mother.

Immunization.—Thus far the use of serum therapy has accomplished little in combating an established anthrax, the sera thus far produced being too weak for successful use in human beings and larger animals. On the other hand, successful vaccination has been practised, especially through the efforts of Pasteur and Chauveau, who based their experiments on the fact that after recovery from anthrax infection there exists immunity from reinfection. According to the methods of Pasteur, animals, such as sheep and cattle, are inoculated with weakened virus obtained by cultivating the organism at temperatures of 42° to 43° C. Two viruses are obtained. No. 1 is capable of killing mice, but not guinea-pigs; No. 2 kills guinea-pigs, but not rabbits (Koch, Gaffky, Loeffler). The first vaccine is first inoculated, and after a period of ten to twelve days an inoculation with the second vaccine is performed. Vaccinations of this kind on a large scale have been carried out in France, Hungary, and Russia, and have proven to be of the greatest practical importance.

Pseudo-Anthrax Bacilli.

Bacillus Anthracoides.—This organism was isolated by Hueppe and Wood repeatedly from water and earth. It resembles the anthrax bacillus in morphology and cultural properties. In blood or tissues the organism is said to exhibit greater rounding of the poles than anthrax. It is sporogenous. The growth on potato, bouillon, and milk is similar to that of the anthrax bacillus, but more energetic. Large quantities of material are needed to produce local effects in guinea-pigs. Mice, guinea-pigs, and rabbits injected with cultures of this bacillus are said to be rendered resistant to anthrax. It is possible that this bacillus represents a saprophytic anthrax bacillus of diminished virulence.

Bacillus Pseudanthracis.—Obtained by Burri from South American fodder. Morphologically it resembles very closely the anthrax bacillus. Its growth upon culture media is somewhat similar. It is sporogenous and rapidly liquefies gelatin. White mice are resistant.

Bacillus Sessilis (Klein).—Found in the blood of a cow that had succumbed to anthrax. Morphologically the bacillus resembles that of anthrax, but the spores are elongated and more like those of B. subtilis. Germination is polar as with anthrax. The organism is non-pathogenic for guinea-pigs.

Bacillus Anthracis Similis.—Obtained by McFarland from a plate colony, which had probably been subjected to contamination from the air. Morphologically and culturally it shows great similarity with the anthrax bacilli, but is non-pathogenic for laboratory animals.

Group of Tubercle Bacilli.

Small, delicate, asporogenous, non-motile bacilli, staining with much difficulty with anilin dyes, usually requiring to be heated, but which are stainable by Gram's method. When once stained, however, the rods retain the color with great persistence, strong acids being, as a rule, required for bleaching. Not all varieties are capable of growth upon the ordinary culture media, and those which grow do so very slowly. The stained individuals show a marked irregularity of their protoplasm, often appearing as beaded rods, certain individuals of which show irregular swellings, chiefly at the extremities, and simple branchings.

Bacillus Tuberculosis. *Synonyms.*—Bacillus of mammalian tuberculosis; Bacillus kochii; Mycobacterium tuberculosis (Lehmann and Neumann).

Morphology and Cultural Properties.—Delicate rods, occurring singly, more rarely as pairs, and in threads. Often slightly curved

Fig. 29.—Bacillus Tuberculosis. × 1,000. (Sternberg.)

or bent. In the bacilli as obtained from pathological lesions or from cultures simple branching occurs. These branches may be single, and only rarely show more than two subdivisions. The bacillus averages 0.2 to 0.4 μ in breadth and 1.5 to 4 μ in length. Growth upon the ordinary culture media is slow, and takes place only at the temperature of the thermostat. The staining of the organism in cover-glass preparations or in tissues is best accomplished by special methods, the most commonly employed being that devised by Gabbett

(see page 604). Gram's and Weigert's methods are applicable. Bacilli once stained retain the dye with such persistence that treatment with comparatively strong acids does not cause decolorization. This fact has rendered it possible to distinguish the bacillus in mixtures of various microorganisms, the practical application of which is seen in the staining of sputa for the tubercle bacillus. In old culures and also as obtained directly from secretions, the bacilli exhibit pale areas which by some have been thought to be spores. These bodies differ, however, from true spores in being less refractive, showing irregularities of form, and failing to exhibit greater resistance to injurious influences than do the organisms which do not show their presence. Babes has described, in addition, in old cultures, polar and central bodies which stained metachromatically. Coppen-Jones has observed, in tuberculous sputum, bacilli with the swollen ends, which strongly suggested similar formations in actinomyces; and Friedreich, Babes, and Leviditi have described rosettes, resembling the Drusen of actinomyces, observed in rabbits injected with cultures of the organism. Babes observed, as early as 1882, that cultures of B. tuberculosis sometimes showed side branches. In the last few years this branching has been observed and studied by numerous bacteriologists, especially by Metchnikoff, Coppen-Jones, Fischel, and Bruns. Craig[31] in this country has described and figured exquisitely branching forms occurring in sputa. The branching in mammalian bacilli is much less marked than in the avian variety. These peculiarities have led some bacteriologists to class the tubercle bacillus with the higher fungi (hyphomycetes), and by Lehmann and Neumann the various forms are grouped under the name of mycobacterium. Kruse considers its relationship to the streptothrices as close, while A. Fischer regards the appearances described as evidence of involution. As early as 1892 Klein described mycelia in cultures.

FIG. 30.—Bacillus Tuberculosis in Sputum. × 1,000. (Baumgarten.)

Tubercle bacilli resist drying for many months, whether kept at the ordinary temperature or in the thermostat. They resist putrefaction for a long time. According to Perrando, the bacilli are still demonstrable by staining methods in decomposing fluids up to the fortieth or even the sixtieth day. Cold has no influence upon them, and in the dried state the organisms may be heated for hours to 100° C. without being destroyed, whereas in fluids they are more quickly killed at lower temperatures (at 60° C. in forty-five to sixty

minutes; at 70° C. in five to ten minutes, Forster). Pure cultures exposed to the direct rays of the sun are soon killed, and diffuse daylight is destructive in from five to seven days (Koch). They may be subjected to freezing and thawing for months, and yet still remain capable of producing tuberculosis in rabbits (Eichborn). Sputa and other discharges may be destroyed by carbolization (three per cent.) after an exposure of about twenty hours. Cellulose is contained in the membrane of the bacillus. The organism grows very slowly or not at all upon ordinary gelatin and agar media, the best medium consisting of gelatinized blood serum. According to Theobald Smith, the serum derived from the dog and coagulated at a low temperature, so as to present a soft surface, is best adapted for a culture medium. Glycerin-agar gives the next best results. Potato is also useful.

Blood serum.—The first growth can be made out with the aid of the microscope on the sixth day, and is apparent to the naked eye by the tenth to the fourteenth day. The colonies are pale, dry, and crumpled. Under low magnifying power the edges are irregular and present S-like forms, which are found to consist of parallel rows of rods. The medium is not liquefied. It is a common experience that cultures made directly from tuberculous foci frequently fail to develop, only a small proportion of many tubes giving positive results. In order to obtain a growth with more certainty, bits of tissue, which include tuberculous foci, especially the most recent, are torn from organs and transferred to the solidified serum. Very little crushing, if any, is desirable, nor should the bits of tissue be rubbed into the surface. After a stay of several weeks in the thermostat the tubes are removed, and the particles of tissue moved about. Prompt appearance of growth now takes place within a week (Th. Smith).

Glycerin-agar.—The earliest colonies are small and present a crumpled surface, the outlines being irregular. The color is white or yellowish-white, the colonies being somewhat elevated from the surface. After the end of three or four weeks lobulation appears, extending from the centre to the periphery. As the colonies grow older, the intensity of the color increases. According to Kitasato, in rare instances the surface of the growth may present a moist appearance.

Potato.—Upon the surface of this medium growth takes place slowly in the form of crumpled yellow fragments, which are usually separate and distinct, and project considerably from the free surface. Growth is well advanced at the end of the third week. In order to facilitate growth, free access of air is required, while at the same time the medium is to be preserved from desiccation. Sander finds an acid reaction of the medium to be most favorable to growth, and po-

tato-mush, to which four per cent. of glycerin has been added, gives a better quality of medium.

Bouillon.—This fluid medium containing four per cent. of glycerin affords a good growth. The development takes place upon the surface in the form of a thick, often wrinkled membrane, from which particles may become detached and fall to the bottom of the vessel or tube. The bouillon remains clear. Growth also takes place in proteid-free fluids containing glycerin.

Pathogenicity.—The bacillus tuberculosis has been found regularly in association with pathological processes in man and animals, and also in external nature. It has been demonstrated in the dust of houses, especially of chambers occupied by tuberculous individuals, in the sweepings from the street, and from railroad cars, and more rarely in the air. The milk of tuberculous cows frequently contains the organism. Probably as a consequence of its presence in the dust disseminated in the air, the bacillus may be found, as shown by Straus, in the secretions from the nose of healthy persons, especially of nurses and physicians who come in frequent contact with the tuberculous individuals. On the other hand, in large numbers of men and animals who during life have shown no signs of tuberculosis, completely healed areas, tuberculous in origin, are demonstrable at autopsy, statistics giving latent or healed tuberculous lesions in as high as sixty per cent. of human beings.

The tubercle bacillus is the acknowledged cause of tuberculosis in human beings and animals, and of the manifold lesions occurring in the disease. We owe to Villemin (1865) the demonstration of the inoculability of tuberculosis and its virulent and contagious character. The demonstration of the specific microorganism was made by R. Koch, in 1882, who succeeded in cultivating it for the first time, and by Baumgarten, who simultaneously and independently demonstrated the bacilli in sections of tuberculous tissue. In human beings tuberculosis is most common in the lungs, these organs being usually the primary seat of the disease. The intestine and skin are sometimes the first portions of the body to be invaded by the tubercle bacillus. The bacilli are most frequently inhaled into the bronchi or air cells, and being deposited there develop locally and produce tuberculosis. At times, however, when they have been carried to the bronchial lymph glands, where they produce tubercles, the lungs may entirely escape. That invasion is possible through inhalation of tuberculous material was shown by Koch in his experiments on rabbits, guinea-pigs, and other animals. Should the infectious material be taken into the mouth, deposition may take place upon the mucous membrane of that cavity, or the material containing the

bacilli may find its way into the stomach and intestines. From deposition upon the tonsils, and perhaps other portions of the buccal cavity, the bacilli may be carried into the cervical lymphatic glands.

The œsophagus is rarely the seat of tuberculosis, although instances have been reported of direct and indirect inoculation of this structure (Cone[32]). Localization may take place upon the mucous membrane of the stomach, and it is common in both the large and small intestine. Primary tuberculosis of the intestine is generally from infected food, the most usual carrier of the organisms being infected milk. A secondary tuberculosis of the intestines is much more common, and is rarely absent in cases of advanced pulmonary tuberculosis, the infection in this instance arising from the swallowing of tuberculous sputum derived from the lungs. The tonsils in these cases, even when they show no macroscopic changes, in sections are frequently found to contain tubercle bacilli and tubercles.

In human beings, tuberculosis has been demonstrated in all of various tissues of the body. Its manifestations are numerous and vary much in individual cases. The organism is capable of producing proliferation of tissue and extensive inflammatory exudations. The serous membranes, the meninges, the solid internal organs, bone, articular surfaces, muscle, connective tissue, epidermis, are all subject to its invasion. The most characteristic lesion is the tubercle—the so-called miliary tubercle. It consists, according to the studies of Baumgarten, primarily of proliferated cells, which are of an epithelioid type. These cells may originate from any of the fixed elements, epithelium, connective tissue, etc. Multinucleated giant cells are commonly present in the tubercles. In the early stages fibrin may make its appearance (Falk), but this substance is more abundant and is more constantly present in degenerated tubercles. The tubercles exhibit a tendency to undergo degenerative changes which, when associated with the presence of molecular fat, produce the condition known as caseation. The caseous material contains the bacilli in a living state. Not only do the newly formed cells of the tubercle suffer in this process, but also the proper tissue elements undergo necrosis, and the blood-vessels in the region are destroyed by thrombosis, by an obliterating endarteritis, or by necrosis. This necrotic material, when thrown off, as may happen in the lungs, stomach, intestines, or elsewhere, leaves behind ulcers or cavities. In the lungs and in the intestines the disintegration of the necrotic material is much facilitated by the invasion of secondary bacteria, the pyogenic cocci. In the lungs the micrococcus tetragenus also acts in the same way. In addition to the growth of tubercles inflammatory exudates are caused by the tubercle bacillus. These may be

found in the serous membranes, in the lungs, and elsewhere, and also exhibit a tendency to undergo caseation. The tuberculous pneumonias are usually produced by the aspiration of softened tuberculous material containing bacilli and toxic products into distant parts of the lung, where it sets up rapid multiplication of the lining cells of the alveoli, and gives rise to an exudate from the blood-vessels, which soon undergo caseation and ultimately break down, producing large cavities. Another mode of transformation of tubercles consists in a fibroid metamorphosis. The protoplasm alters in staining-properties, becomes hyaline, and eventually a fibrillated material makes its appearance between the cells. About such tubercles scar-like fibrous tissue develops. In old tuberculous foci, especially in those which have undergone caseation, there is a tendency to the deposition of lime salts. This is marked more particularly in the lungs, where such concretions may remain the sole survivals of old tuberculous foci. The lime-crusted material may still continue to contain living tubercle bacilli. Tubercles are usually multiple, but single ones—the so-called solitary tubercles—occur in certain situations, more especially in the central nervous system. These may appear as tumor masses the size of a hazelnut, or even of an orange.

Tuberculosis also appears as a natural disease among domesticated animals, especially cattle. It is estimated that as much as thirty-five per cent. of slaughtered cattle have been tuberculous (Lehmann and Neumann). The lesions may appear in the lungs, udder, and serous membranes. In the form known as "pearl" disease (Perlsucht), especially common in cattle, large nodules, many of them calcified, occur in the peritoneal cavity. In some localities swine also are found to be tuberculous, but sheep, goats, horses, dogs, and cats are much more rarely the subjects of the natural disease. Birds are not uncommonly affected (*vide infra*).

Tubercle bacilli are pathogenic for a large group of experimental animals, the most susceptible being the guinea-pig. These animals die after inoculation with the smallest quantities of living tubercle bacilli, whether in the form of pure cultures or contained in tissues, secretions, etc. The most rapid development takes place after intraperitoneal injection; if the number of bacilli introduced be at all large, death may occur in from ten to twenty days. The omentum is rolled up into a kind of ball which contains firm caseous masses rich in tubercle bacilli. There is little or no fluid present in the peritoneal cavity, although an excess is found in the pleural cavities. The spleen is swollen, and contains, as does the liver, tubercle bacilli and microscopic tubercles. When the material inoculated is poorer in bacilli, the disease progresses more slowly, the animal lives longer, and at

autopsy macroscopical tubercles can be seen in the spleen, liver, lungs, and elsewhere. Subcutaneous inoculation produces a nodule usually after about a week, which breaks down and remains as a caseous progressive ulcer, while the neighboring lymph glands become swollen, and may reach the size of hazelnuts. An irregular febrile movement appears. The animal loses weight, and death takes place in from four to twelve weeks. If only very small amounts of bacilli be injected, the nodule at the local seat may heal, and death be delayed for a longer time. At autopsy the lymph glands are found to be enlarged and caseous; the spleen is greatly increased in size and contains large tuberculous nodules. The lungs show numerous but much smaller tubercles. In these more chronic experimental infections the number of bacilli in the lesions is much smaller.

Rabbits are also susceptible, but far less than guinea-pigs. The introduction of bacilli or tuberculous material into the anterior chamber of the eye produces a fatal infection. A tuberculous iritis develops, and from this source the bacilli pass through the lymphatics, involve the nearest lymph glands, and eventually produce a generalized tuberculosis that causes death at the end of weeks or months.

Subcutaneous inoculations are less active, especially when small numbers of bacilli are introduced. The intravenous and intraperitoneal injections are more successful, leading to generalized tuberculosis. The tuberculous nodules in the rabbit are usually small; the spleen and liver are not so profoundly affected as in the case of guinea-pigs, whereas the kidneys may show much larger nodules. Bacilli of weaker virulence give rise to large nodules in the lung, which may eventually become hollowed out, leaving cavities. This excavation, however, as has been shown by the experiments of Prudden, is greatly accelerated if pyogenic organisms gain entrance to the tuberculous tissues. The introduction of cultures beneath the dura mater and intravenous injections produce tuberculosis associated with peculiar morphological alterations of the bacilli, so that forms are met with which bear much resemblance to actinomyces (Friedreich, Babes and Levidíti). The injection of dead tubercle bacilli into the lungs of animals produces tubercles which, however, do not degenerate (Prudden). Still other animals are susceptible, such as the field-mouse and cats, and if the doses are sufficient, white mice, rats, dogs, canary birds, etc. The inoculation by Bollinger of tuberculous material derived from man into calves led to the production of pearl disease in the abdominal cavity. The early positive experiments upon birds made with human tubercle bacilli are now recognized as of doubtful significance. Koch succeeded in a few instances, by intraperitoneal injections, in causing infection in hens and pigeons, but later experimenters

(Maffucci, Kruse, and Pansini) have not been able to confirm these observations. On the other hand, Pansini and Kruse have produced experimentally a local infection of the comb. According to Kruse it is probable that among the larger birds only the parrot is susceptible to mammalian tuberculosis. Tuberculous infection may be produced by feeding animals with tuberculous material or bacilli. The local reaction tends to appear in the mesenteric glands rather than in the intestines (Wesener). There is experimental evidence, which is borne out also by our knowledge of the natural disease, to prove that infection may take place through the intestinal tract without localization upon the mucous membranes, the lymphatic glands of the abdominal cavity being first affected. Infection may also take place by inhalation, as was shown by Koch in the case of rabbits, guinea-pigs, rats, and mice. Infection by inhalation can be produced most successfully by atomizing watery suspensions of the bacilli, the dried tuberculous material conveyed in dust being less effective. The inhalation forms of tuberculosis of the lung produced experimentally do not agree with the most common forms in human beings, inasmuch as cavities are rarely formed, the process taking the form of a lobular caseous pneumonia. The post-mortem tubercles (Leichentuberkel) are due to cutaneous inoculations with material containing tubercle bacilli. Armanni and Baumgarten have produced similar lesions in guinea-pigs and rabbits experimentally.

Modes of Infection.—Tuberculosis of the lung, except in rare instances, is to be regarded as an inhalation form of the disease, the infectious material coming from the outside and being brought immediately into close relation with the pulmonary tissue. The lung is by far the commonest seat of the primary lesions of the disease and forms the portal of entry from which infection is conveyed to other parts. Primary intestinal and peritoneal localizations of tuberculosis are relatively unknown in adults, but not very uncommon in young children, the infectious material probably gaining access with the food. Tuberculosis of the skin—lupus—is an example of cutaneous infection. There can be little doubt that, all things considered, tuberculous sputum is the chief agent in the dissemination of the bacilli. The particles may be dry and in the form of dust, or consist of droplets of sputum expelled in coughing. Flügge holds that the danger to those around arising from sputum expelled by a consumptive in coughing decreases with the distance from the patient. At 50 cm. numerous particles are encountered; at 150 cm. they are much fewer. Naturally any such rule has wide variations according to the strength of the cough and other circumstances. The wide dissemination of the tuberculous virus is shown by the large numbers of

individuals who succumb to tuberculosis, or show the existence of latent or healed lesions in the internal organs, or in whom the presence of living bacilli upon the mucous membrane (nose, tonsils) is demonstrable. At the same time the very number of individuals of the latter classes emphasizes the fact that the mere presence of the tubercle bacilli is insufficient to cause infection or produce pronounced lesions in those who are exposed to them. It is therefore held by many that in addition to the presence of the virus there must exist a predisposition, which is coincidentally operative in those individuals who develop serious and fatal forms of the disease. In part this is supplied by depressed physical and perhaps also by mental states, or by the existence of other pathological conditions (persistent catarrh, diabetes, cirrhosis) which favor the development of the tubercle bacillus, or, indeed, of other pathogenic micro-organisms.

The origin of primary foci of tuberculosis in the internal organs in connection with the lymph glands, joints, bones, or other tissues, together with rarer forms of tubercles, is somewhat more difficult to explain. From what has already been said, it seems highly probable that infection of the cervical lymphatic glands can take place through the buccal mucous membrane without any necessary pre-existence of a tuberculous lesion. Tuberculosis of the mesenteric glands is explicable on the basis of Wesener's experiments, in which the bacilli were absorbed from an intact intestinal tract. The infection of bone is somewhat more difficult to explain, although it is possible that bacilli may be present in the circulation without producing lesions in the tissue surrounding the point of entry, and may be carried by the blood current and be deposited in the bony structures, as has been shown in the experiments of Zagari on the dog. Primary tuberculosis of the female genitalia has been observed. The mode of infection is not always clear, but the origin from the semen of tuberculous males may be suspected in certain cases. Tuberculosis of the uterus and Fallopian tubes is a recognized cause of tuberculous peritonitis in females. Whether more distant parts may be infected from this source is unknown.

To explain the localization in the internal organs, it has been held, especially by Baumgarten, that tubercle bacilli may be transmitted directly from parent to offspring. Observations upon animals and upon man, partly natural and partly experimental, prove conclusively that the tubercle bacillus may pass the placental barrier and be deposited in the fœtus, in which the lesions of tuberculosis may be developed. Numerous instances of fœtal tuberculosis in calves have been reported by Johne, Czokor, Malvoz and Bovwier, and others, while examples in human beings have been observed by Birch-

Hirschfeld, Schmorl and Kockel, and others. Experimental proof of the transmission of tubercle bacilli from mother to offspring has been brought by de Renzi and A. Gärtner. Experiments upon guinea-pigs, mice, rabbits, and canary birds have shown that the infection of the fœtus may take place either from a generalized tuberculosis or from local lesions. The number of microorganisms in the fœtus is usually small, and frequently of a whole litter not more than one or two of the fœtuses show an infection. The length of time that bacilli may remain alive without producing lesions is not known, so far as regards the mammalian variety. Maffucci, who inoculated hens' eggs with avian bacilli, observed a latency of some weeks or months in the incubated chickens. The positive results of fœtal infections obtained in animals would indicate a placental rather than a germinal origin. The fact that the semen of tuberculous males contains tubercle bacilli (Jani, Jäckh, Walther, and others) opens up the possibility of infection of the ovum at the time of its fecundation. There is thus far, however, no experimental evidence to support such an assumption. In Gärtner's experiments infection of the fœtus from this source did not take place, the chief danger, as shown by these results, lying in the risk of localized infection of the female genitalia from the tuberculous semen. Hauser,[33] in a recent critical review of the theory of bacillary inheritance of tuberculosis, concludes that it is insufficiently grounded. He found the evidence which has thus far been adduced so contradictory as to fail entirely to supply a satisfactory explanation of so-called inherited tuberculosis. In the instances of fœtal infection the localization of the lesions has been entirely different from that ordinarily met with in tuberculosis. Thus in nine cases of congenital human tuberculosis the liver was involved five times, while in five instances in which tubercle bacilli were present in the absence of anatomical lesions of tuberculosis, the organisms were contained chiefly in the liver and the periportal lymphatic glands, or in the liver only. In sixty-four instances of congenital tuberculosis of cattle, the liver and portal glands were affected in fifty-two. According to these figures the liver and portal glands, as the chief seats of the disease, were involved in eighty per cent. of the congenital cases, whereas in the disease as it ordinarily occurs these organs are very rarely affected. Generalized tuberculosis in extrauterine life is the result of the dissemination of tubercle bacilli by the blood current, the main source of the virus being infected sites in the lung, where, as has been pointed out by Weigert, the tuberculous process penetrates into blood-vessels, liberating tubercle bacilli, which find their way first into the pulmonary vessels and later are carried to all parts of the body by the general systemic circulation. As a conse-

quence, bacilli are deposited in various organs, the spleen, liver, kidneys, brain, serous membranes, bone, thyroid gland, pancreas, or elsewhere, and in these different situations tubercles develop. More rarely one or more tuberculous lymphatic glands, particularly about the bronchi, become adherent to a blood-vessel and rupture into it; or it may happen that material containing bacilli is carried with the lymph from the thoracic duct into the right heart, whence it may readily be distributed throughout the body. Finally the thoracic ducts may be the seats of developments from which the infectious material is carried to the heart.

Avian Tuberculosis.—Maffucci, Straus, and Gamaleïa have shown that the bacilli of the tuberculosis occurring in birds present definite morphological, cultural, and pathogenic properties, by which they can be distinguished from the bacilli of mammalian tuberculosis. That the bacilli of avian tuberculosis may, however, be present in human beings and other mammals has been shown by the studies of Kruse, Fischel, and Pansini.

The bacilli of avian tuberculosis, while resembling in form, size, and staining-properties those of human tuberculosis, show a greater tendency to the production of swollen and expanded ends and branches. Upon artificial culture media the differences are still more striking, and development is more rapid, the growth presenting a moist, smooth, or wrinkled surface, which, upon being touched by the platinum needle, appears soft and slimy. Upon glycerin-agar perceptible growth is obtained at the end of eight days. The culture sometimes assumes a blackish, reddish, or citron-yellow color. According to Maffucci the temperatures suitable to the development of the avian bacillus are from 35 to 45° C., for the mammalian variety from 30 to 40° C. Cultures of the former remain alive for from one to two years, and are more resistant to high temperatures (65 to 70° C.) than the mammalian organism. Animal experiments give still more convincing proof of the existing differences. The most susceptible animal is the hen, which succumbs to intraperitoneal inoculations in from one to several months. Subcutaneous injections are less certain, while positive results may be obtained by inoculation into the trachea or into the blood-vessels. Inoculation into the comb produces localized lesions. Of the internal organs the spleen and liver show the most marked changes, which to the naked eye would appear to consist merely in an enlargement. Microscopically, however, masses of bacilli are found in the tissue, and proliferated cells forming tuberculous nodules are usually to be made out. Ducks, pigeons, pheasants, and other birds are susceptible. Mammalia, on the other hand, are refractory, or react merely by local lesions, and

occasionally, provided that large quantities of material have been inoculated, by a generalized tuberculosis. The natural disease in birds is commonest in poultry, which in certain regions may be affected in large numbers. The lesions are located chiefly in the liver, the spleen, less often in the peritoneum, intestine, and ovaries, and only seldom in the lungs. Maffucci and Baumgarten have shown that, if fecundated eggs are inoculated with avian tubercle bacilli, and then incubated, the young may die after the lapse of many months; in these cases lesions are found more particularly in the liver and peritoneum. The transmission of the bacilli from mother to egg was proven by the experiments of Gärtner (*vide supra*). Nocard incubated bacilli of mammalian tuberculosis, enclosed in celloidin sacs, in the peritoneal cavity of the cock, and claims that by this procedure he was able to impart to them the biological characters and virulence that belong to the bacilli of avian tuberculosis.

Bovine Tuberculosis.—Hitherto the absolute identity of tubercle bacilli infecting mammalia has been generally assumed, and, moreover, the assumption has been used as a basis for the enactment of sanitary measures having for their object the prevention of any transmission of tubercle bacilli from animals to men. Theobald Smith has presented a comparative study of human and bovine tubercle bacilli, which seems to indicate differences in morphology, in cultural behavior, and in pathogenic effects between the human and certain animal bacilli, including those obtained from cattle. The characteristics which distinguish the bovine variety are stated by Smith to be found morphologically in the invariably short, straight form, and in the greater resistance of this type to the modifying influences of culture media; and biologically, in a greater resistance to artificial cultivation, and in greater pathogenic activity towards rabbits, guinea-pigs, and cattle. The discussion of the question is still in the early stages, although there have already been collected not a few examples of the undoubted transmission of bacilli of the one order to the proper host of the other species. Tubercle bacilli obtained from human cases have been found by Bollinger to give rise to pearly disease when injected into the peritoneal cavity of cattle. Sydney Martin fed calves with sputum containing tubercle bacilli, and found lesions in the intestines, chiefly in Peyer's patches. Smith observed that the lesions produced in cattle by the inoculation of sputum bacilli were local and restricted in character. They were not found beyond the place of deposit, or in the lymphatic glands to which this was tributary, except in the case of one animal (one out of five). Moreover, in cattle the lesions appeared chiefly as granulation tissue, while tubercle formation only rarely occurred. Smith

concludes that the sputum bacillus cannot gain lodgment in cattle through the ordinary channels, as, for example, the respiratory mucous membrane. On the other hand, there are reports indicating the transmission of the bovine variety to human beings.

Fish Tuberculosis.—Bataillon, Terre, and Dubard have described a tumor of the abdominal wall in the carp, containing many giant cells and bacilli; the latter resembled morphologically the tubercle bacillus, and grew best at temperatures of from 23° to 25° C. The growth was rapid, appearing in bouillon at the end of three or four days. Growth was also obtained upon potato, gelatin, and other media. The organisms branched dichotomously. Intraperitoneal inoculation in the case of the carp proved to be without effect, so far as the production of tubercles is concerned. Frogs reacted, but only once were nodules found in the lung. In other cases the bacilli only were found in the organs. In a later publication, Kral and Dubard [34] claim that they have converted the bacilli of human tuberculosis and of avian tuberculosis into a form similar to that obtained from the carp. This was effected by passages through fish, frogs, lizards, and so on. The original infection of the carp is supposed to have been due to human bacilli which had gained entrance into the waters of the stream.

Toxic Products.—Cultures of the tubercle bacillus contain certain metabolic products of growth, which, when extracted with glycerin and concentrated, appear in commerce under the name of tuberculin. Analyses of this substance have shown it to be a complex structure. It is free from crystalline alkaloidal principles and ptomains. Alcohol produces the formation of a precipitate, which contains a toxic ingredient. Thus far this substance has not been obtained in a pure state and its chemical nature is unknown. Various attempts have been made to purify it. One product obtained is known as tuberculocidin, a fractional precipitate with alcohol prepared by Klebs. Tuberculin had been vaunted as a promising cure for tuberculosis. It is now no longer used for this purpose, although, on account of the reaction which it produces in tuberculous individuals, it is still employed for diagnostic purposes both in human beings and in animals. It is possible to lengthen the life of tuberculous guinea-pigs by the cautious injection of tuberculin. These animals after being submitted to inoculation of virulent cultures have been kept alive under this treatment as long as eight months. The study of tubercles after the injection of tuberculin has shown that they become the seat of inflammatory reactions, which in the case of foci situated externally may lead to their separation and removal, whereas those in the internal organs are not favorably affected. All attempts thus far made to produce a serum that would be curative for tuberculosis have failed.

Diagnosis of Tuberculosis.—The tubercle bacillus stains with some difficulty, requiring high temperatures, long immersion, and the addition of some mordant (alkali, anilin oil, carbolic acid) to the stain. But having once taken up certain coloring-matters it holds them with great tenacity. This feature makes it possible to differentiate the tubercle bacillus when admixed with other organisms in secretions, excretions, dejecta, etc. The procedure will vary somewhat with the material. Sputum is spread upon cover-slips, allowed to become air dry, and then stained (see page 599). It is best to select the small flakes of muco-pus from the sputum. In the case of urine it is best to take a centrifugalized specimen, a sufficient quantity of the precipitate being collected and spread upon a cover-glass. The same holds good for milk and other fluids, as, for example, those obtained by aspiration from the pleura, peritoneum, spinal canal, or joints. The organisms are somewhat more difficult to demonstrate in fæces, but in fluid dejecta mucous and purulent flakes are to be selected for study. Failure to demonstrate tubercle bacilli in any of these preparations does not prove their absence. The number of organisms may be very small, so that they may be readily missed in the microscopical examination. To obviate this difficulty, when the microscope has failed to demonstrate the organisms, the suspected material may be inoculated into a guinea-pig, subcutaneously or intraperitoneally. When other organisms are mixed with the material, it is best not to inject it into the peritoneal cavity. The great difficulty of obtaining cultures of the tubercle bacillus directly from the animal body practically precludes the use of that method for the purposes of diagnosis.

It is customary to accept as tubercle bacilli microorganisms remaining stained after the treatment with acids and presenting the morphology of the tubercle bacillus. Not all such organisms, however, are certainly the B. tuberculosis. In human beings care should be exercised to avoid the confusion of another acid-resisting bacillus, B. smegmatis (page 722), which is normally present upon the surface of the body. The chief dangers of confusion are found in the study of urine and fæces because of the presence of the latter bacillus in the smegma. A. Fraenkel and Pappenheim have drawn attention to the occurrence of B. smegmatis in human sputa. Pappenheim recommends the following method of differentiation:

1. Stain in carbol fuchsin.

2. Decolorize without washing by immersing from three to five times in a solution composed of absolute alcohol, 100.0; corallin, 1.0; methylene blue to saturation; glycerin, 20.0.

3. Wash, dry, and mount in balsam. The tubercle bacilli remain red; the smegma bacilli assume a blue color.

Bacilli Resembling B. Tuberculosis.—Examination by Rabinowitsch and others of commercial butter have revealed the existence of a group of bacteria resembling in morphology and staining-reactions the bacillus of tuberculosis. They are widely disseminated, and under favorable circumstances give rise to pathological states easily to be confused with tuberculosis. Rabinowitsch examined thirty specimens of butter obtained in Berlin and fifty in Philadelphia. From samples of butter which had been kept for from twelve to twenty-four hours in the thermostat, injections were made into the peritoneal cavity of guinea-pigs. The animals were killed at periods varying from three weeks to three months. In no instance were tubercle bacilli found. On the other hand, twenty-three of the samples (28.7 per cent.) produced in guinea-pigs macroscopical and microscopical changes which could easily be confounded with tuberculosis. Cultures from the peritoneal cavity of these animals gave growths in from two to three days. These consisted of thick, moist, creamy membranes, which, as they became older, assumed an orange or copper color. The cultures in bouillon looked very much like those of B. tuberculosis. The tubercles differed in their histology from those caused by B. tuberculosis in that they showed a distinct tendency to purulent softening, and, as a rule, lacked the giant cells. Similar bacilli have been obtained from manure and timothy-grass. They show, in cover-glass preparations, swollen extremities and occasional branches. According to G. Mayer, when injected alone into the peritoneal cavity of guinea-pigs they do not produce pseudotuberculosis. When, however, they are injected along with butter, opportunities are afforded for their development, and they set up a fatal peritonitis, associated with appearances of pseudotubercles. The injection of butter alone has no such effect. Epithelioid and giant cells were observed by Mayer in the tubercles, which also contained large numbers of the bacilli.

Bacillus Lepræ.—First observed by A. Hansen and A. Neisser in the tissues of leprous individuals. The organism has since been found by bacteriologists wherever leprosy has been studied.

Morphology.—The bacilli resemble tubercle bacilli, although they are somewhat shorter and of a more uniform thickness. They are resistant to acids, although staining somewhat more easily than B. tuberculosis. When freshly obtained from lesions, they stain with ordinary anilin dyes, but tissues which have been preserved in alcohol are best stained by the method used for demonstrating B. tuberculosis. Positive results are also obtained with Weigert's and Gram's methods. The stained bacilli exhibit uncolored vacuoles. Rods with swollen ends have been observed, and branching has been

described (Babes, Czeplewski, and others). Babes has described a form resembling the rays of actinomyces. Numerous attempts have been made to cultivate the leprosy bacillus, but without success. Thus far all of the statements to the contrary effect, of which there are many in the literature, have upon close examination been found to rest upon error, inasmuch as other microorganisms, some of which were closely related to B. pseudodiphthericus, have been obtained.

Pathogenicity.—B. lepræ, although it has never been cultivated outside the body of human beings, and no inoculation experiments of a satisfactory nature are at hand, is nevertheless regarded as the cause of leprosy. The organism occurs, and usually in enormous numbers, in the lesions of human beings suffering with leprosy, with the exception of certain cases of tubercular and macular anæsthetic leprosy. It is highly desirable, on account of the somewhat variable behavior of the leprosy bacillus with respect to stains, that hereafter in all negative cases the method of staining employed should be given. The bacilli may be found in cases of lepra, especially in the nodules of the skin, but also in the conjunctiva, cornea, mucous membrane of the mouth and larynx, lymph glands, interstitial tissue of nerves, central nervous system, testicle, spleen, liver, kidneys, and seminal fluid. They also occur in the circulating blood, either free or enclosed in leucocytes. In histological preparations the bacilli may be found within the lumina of blood-vessels, frequently filling the endothelial cells. They have, moreover, been found in the intestines, the lungs, and in sputum.

The mode of infection is still a question of doubt. There is much evidence that the skin offers the chief portal of entry, inoculations occurring directly into the exposed parts, as, for example, the lower extremities in tropical countries, where people go unshod (Arning). Schäffer exposed clean slides on tables and floors in proximity to lepers whom he caused to read aloud, and found that the atomized drop of buccal secretion, which would sometimes float about in the atmosphere for hours, contained leprosy bacilli. He concluded that the inoculation might be spread in this way through the air. Babes has shown that the bacilli may be thrown off by the skin and mucous membranes, and he also demonstrated their presence in milk. He found large numbers of bacilli in the nipple of a nursing woman who had infected her sucking child. The part played by heredity seems, with our present knowledge, to be a very minor one. In the United States leprosy prevails to a very limited extent. Dyer, of New Orleans, has been able to collect three hundred authenticated cases which have occurred since 1785. He claims to have seen personally one hundred

and eight cases in the last few years. Sporadic cases appear from time to time in other parts of the United States. The writer has observed two instances, both in women of the Caucasian race.

Bacillus Syphilidis.—This organism was observed simultaneously by Lustgarten and Doutrelepont, who found it in syphilitic lesions. Their observations have since been confirmed by numerous investigators. The bacilli resemble in size tubercle bacilli; they are often bent and assume S-forms. They show irregular swellings, contain unstained vacuoles, and tend to break up into granules. In the tissues they appear either singly or in groups, which may be intracellular or lie free. In staining they resemble the tubercle bacillus, but are much more easily decolorized by acids. Immersion in the decolorizing fluid should be rapid, and should not exceed from three to five seconds. Lustgarten decolorized by means of alcohol, permanganate of potassium, and sulphuric acid. Cultivation of the bacillus has thus far failed, and the inoculation of syphilitic lesions and secretions has also given negative results in animals. In human beings inoculations have been successful with the exception of those from tertiary lesions. The bacilli have been found in the initial lesions and papules, in flat condylomata, and also in gummata. The relation of the bacilli to syphilis is still problematical. The organisms are present in small numbers as a rule, and have thus far not been cultivated. On the other hand, the absence of all other microorganisms may be taken as indicating the possible etiological rôle of Lustgarten's bacillus. The danger of confusing this organism with the smegma bacillus is not much to be feared if the distribution of the smegma bacilli be remembered, and also the difference in the staining-reaction of the two organisms. The differential diagnosis between the bacillus of Lustgarten and B. tuberculosis is made by the behavior towards acids, while, as regards the smegma bacillus, the resistance of the stained organism in syphilitic lesions to alcohol usually suffices. In doubtful cases in which B. tuberculosis is supposed to be concerned, animal experiments should be carried out.

Bacillus Smegmatis.—Obtained by Alvarez and Tavel, Matterstock, Klemperer, etc., from the preputial secretion and in the folds of the groin, in the region of the anus, and elsewhere. The organisms are especially numerous in the fatty secretion of these parts, lying in masses either upon or within the epithelial cells. In form the bacilli resemble both B. tuberculosis and B. syphilidis. They show, however, greater irregularities in size, etc. Staining is difficult; the dye once accepted, is given up with difficulty to acids, although more readily to alcohol. After alcohol bleaching certain individuals may still retain the stain. Inoculation of animals is without effect. Cul-

tivation is difficult, but has succeeded upon coagulated hydrocele fluid (Doutrelepont and Matterstock) and in milk (Czaplewski).

Spirillaceæ.

Spirilla are long, spirally bent or corkscrew-like cells which possess generally a single flagellum and rarely two polar flagella. They are asporogenous, although, according to Hueppe, arthrospores occur.

Spirillum Choleræ Asiaticæ. *Synonyms.*—Vibrio choleræ asiaticæ; Koch's comma bacillus; Bacille virgule.

Morphology and Cultural Properties.—Bent rods averaging 0.4 μ in width and 2 μ in length, the ends of which do not lie upon the same plane. The bends are sometimes delicate, at other times pronounced; in the latter case hemispherical forms appear. The union of two vibrios gives rise to S and E forms. Under unfavorable conditions of growth, as for example in the presence of insufficient oxygen or proteid, corkscrew forms in which the individual members are difficult to make out are obtained. Old cultures show various involution forms. The organisms show a rapid and screw-like motility, due to the possession of one, sometimes of two polar flagella, which have an undulating corkscrew-like form. Staining is accomplished with the ordinary dyes, but not by Gram's method. The best results can be obtained through the use of diluted carbol fuchsin (1:10), which is permitted to act for some minutes. Growth takes place rapidly when air gains access; under anaërobic conditions it is far slower. The optimum temperature is about 37° C., although growth is still active at 22° C., and takes place slowly at 8° C.

Fig. 31.—Spirillum Choleræ Asiaticæ. × 1,000. (Koch.)

Gelatin.—The colonies at first are yellowish-white and round. After from twenty-four to thirty-six hours liquefaction begins, and the colonies tend to sink into the depth. The ring of liquefaction at first is clear, but after a time becomes cloudy, owing to the invasion of spirilla. The young colonies, sixteen to twenty-four hours old, when magnified sixty times, appear as small yellowish-white, round, coarsely granular discs, with more or less granular edges. As they grow older they become more granular, and eventually lobulated. In the most characteristic stage they look as if they had been dusted over with splinters of glass. After liquefaction the edges are irregu-

larly granular, and sometimes show hair-like outgrowths; but the whole colony may remain compact, surrounded by the liquefied medium. The variations in the form and appearance of the colony are very great. In gelatin stabs growth is first thread-like, and does not become characteristic until the end of from twenty-four to thirty-six hours. Liquefaction now begins at the surface, and a small depression is formed, which, as it increases in size both laterally and in the depth, is occupied by a bubble of air. Liquefaction continues in the depth, follows a funnel-shaped course, until finally the whole medium is dissolved. The liquefaction zone remains clear, or it may contain small fragments of the growth which tend to settle to the bottom and present a yellowish-white color. Cultures which have been kept for

Fig. 32.—Spirillum Choleræ Asiaticæ; colonies upon gelatin plate, end of thirty hours. × 100. (Fränkel and Pfeiffer.)

a long time in laboratories usually liquefy less perfectly and more slowly than those obtained with freshly isolated organisms. This power may be lost entirely and then suddenly regained.

Agar.—The colonies are less characteristic; they are round, pale brown, and sometimes white in color, with edges which appear smooth to the naked eye. Magnified sixty times the deep colonies are found to be irregularly round, and sometimes whetstone-shaped, while their margins are smooth or very slightly granular. As they become older the color grows more pronounced. Superficial colonies are rounded, pale yellow in color, translucent, and after a time coarsely granular. Agar stabs show only a grayish-white, non-characteristic, thread-like granular growth. On slant agar there appears a soft, glistening, elevated membrane, which after a time assumes a yellowish-brown color. Blood serum is rapidly fluidified.

Bouillon.—At the end of from ten to sixteen hours there is diffuse clouding, and, as a rule, a membrane, often very brittle, develops upon the surface. The cultures recently obtained from an animal body may fail to give the membrane. On the other hand, according to Cramer, a strong alkaline reaction of the medium increases its thickness and compactness.

Milk.—The original cholera cultures of Koch were without effect upon plain milk. More recently cultures obtained from cholera patients have not infrequently caused coagulation. In litmus-milk acid production is observed. The lab-ferment is produced (Sclavo).

Potato.—On slightly acid potatoes growth takes place very slightly or not at all, and only at the thermostat temperature. Potatoes which have been treated with soda solution (0.25–0.5 per cent.) serve as a suitable culture medium. Growth takes place at 20° C. as well as in the thermostat. Upon neutral potatoes the growth is at first of a dirty white or yellowish color, very slightly elevated, but soft and shining; after a time the color becomes of a brownish-red, the growth extending over the entire potato.

Gas is not formed from any of the sugars, although acids, chiefly lactic acid, are produced.

The spirilla may survive in cultures for a whole year, if complete drying is avoided. In sterilized water the duration of vitality is variously stated to be from one day up to a year. Low temperatures, exclusion of light, and the presence of salts favor their preservation. In the waters of brooks and rivers the spirilla usually disappear in from three to eight days. Drying quickly kills the organism, not infrequently in five hours. At 60° C. they are killed in ten minutes. Temperatures of from 5 to 10° C. kill them in from three to eight days. Kasansky, however, has found that four months of a Russian winter is not sufficient to completely destroy the vibrios. Sunlight acts injuriously upon them, and although it does not kill them at the end of seven hours their motility is found to have been lost.

Indol is formed more particularly upon the media containing proteid. At the end of six hours its presence is already demonstrable by color tests. The nitrates in the peptones and commercial salt are reduced to nitrites; hence the addition of sulphuric acid alone gives the cholera-red reaction (Dunham and Bujwid). This is the so-called nitroso-indol reaction of chemists. The reaction is much more pronounced in cultures from twenty-four to forty-eight hours old, but as the nitrite gradually disappears still older cultures fail to give the reaction, unless a nitrite be added. Indol is formed in solid cultures, as can be shown by diffusing a large loop of an agar culture in 10 c.c. of peptone water. A positive reaction is very rarely absent.

Pathogenicity.—In 1883 cholera was epidemic in the Nile delta, and sporadic cases were brought to the several ports of Europe. At this time the German government sent out a commission under the direction of R. Koch to discover if possible the cause of the pest. In Egypt, where the first stop was made, Koch found in the intestinal contents and in the intestinal wall of fresh cases of cholera a well-characterized bacterial species, now recognized as the spirillum of cholera. Further studies pursued in India, the home of cholera, showed that this species was a constant inhabitant of the intestinal contents of persons suffering from cholera. In the earlier and severer cases the organisms were very abundant, whereas they were not obtainable from the intestinal contents of healthy persons or of those suffering from other diseases. Koch at the same time discovered that the spirilla were not present in the blood and other internal organs. In the most acute cases, which were characterized by slight swelling and congestion of the mucous membrane of the intestine, the contents of which were colorless—the rice-water or meal-soup stools—vibrios were present almost in pure culture. When the disease lasted longer the mucous membrane showed greater changes, a mottled reddening, especially at the edges of the follicles and of Peyer's patches being perceptible. In these cases the comma bacilli had penetrated the mucous membrane, and were found in the crypts, in part between the epithelium, and in contact with the basal membrane. In the more superficial parts of the mucous membrane other bacteria were encountered, which seemed to follow in the wake of the spirilla. A third group of cases showed various secondary changes. The lower part of the small intestine was of a dark brownish-red color. The mucous membrane contained many hemorrhages; its surface was sometimes necrotic and covered with diphtheritic membrane. The intestinal contents in these cases were bloody, gangrenous, and of a very foul odor. The comma bacilli were very difficult to demonstrate, whereas many other bacteria of various forms were present. These observations of Koch have been extensively confirmed. Minor variations have been noted. In rare cases, in man as well as in experimental animals, the vibrios may be found in organs other than the intestine —in the lungs, liver, kidneys, spleen, and most rarely in the blood of the heart. The more virulent the organism the greater its tendency to invade the organs. Again, during the prevalence of cholera, it has been found in infected districts that the spirilla may be present in the dejections of healthy persons who show no pathological symptoms whatever. Abel and Clausen examined seventeen persons who were in constant contact with cholera patients; in the stools of fourteen the spirilla were demonstrated. In some cases they persisted for four-

teen days. During the epidemic in Hamburg in 1893 a considerable number of healthy persons were found who harbored the vibrios in their fæces.

Spontaneous outbreaks of cholera do not occur among the lower animals, and with experimental inoculations special precautions are necessary in order that the appearances of the natural disease may be produced. In order to bring certain proof that this vibrio is the cause of Asiatic cholera several tests upon themselves have been voluntarily made by investigators in laboratories. These were carried out in Munich and in Paris. The results to the experimenters were sufficiently severe to indicate positively the pathogenic character of the spirillum and its capacity to produce cholera-like infections. Such experimentation is, of course, to be deprecated; indeed, the occurrence of accidental laboratory infections, one of which ended fatally, furnished the necessary final proof of the specificity of the cholera vibrio and rendered unnecessary any exposure to the risks belonging to voluntary inoculation.

The cholera vibrio, in its living as well as in the dead state, is pathogenic for animals, especially for the guinea-pig, which succumbs to intraperitoneal inoculation of minimal quantities of cultures. The ingestion of cultures usually gives negative results unless, as a preliminary, the precaution be taken to neutralize the gastric contents and control peristalsis. Koch proceeds as follows in producing infection through the mouth: Guinea-pigs receive through a small catheter passed into the stomach 5 c.c. of a five-per-cent. soda solution. After an interval of a few minutes 10 c.c. of fluid containing several drops of a pure culture of the comma bacilli are introduced. The animals after the injection are given a dose of opium (1 c.c. of the tincture to 200 gm. body weight of the animal), which is injected directly into the peritoneal cavity. The effect of the opium is to cause narcosis, lasting from half an hour to an hour, after which the animal seems to all intents and purposes well. After ten or twelve hours, or at the longest on the next day, the animals fail to take their food and look sick. Weakness of the posterior extremities now sets in, the respirations become slow and superficial, the animal shows signs of collapse, and death occurs. At autopsy the small intestine is found to be hyperæmic, and is filled with a watery, colorless fluid containing whitish flakes. An excess of fluid is also present in the stomach and in the cæcum. Microscopical examination of the contents of the small intestine shows almost a pure culture of the comma bacilli. The explanation of this action is simple. The alkali neutralizes the acid of the gastric juice; otherwise the introduced spirilla would be destroyed. The injection of opium causes

temporary cessation of peristalsis, so that the culture reaching the intestine has time to undergo development before it is expelled. This development brings about the symptoms which have been described, and which are chiefly due to the absorption of poisons elaborated by the spirilla. A similar result can be obtained by injecting a small amount of a fluid culture directly into the duodenum through the opened abdominal walls, after which the duodenum is caught with the forceps and held for a short time, so that a temporary paralysis of the muscle is produced. Other microorganisms introduced in these ways fail to produce similar results, excepting in a small percentage of cases.

Thomas, Issaëff, and Kolle have shown that rabbits are susceptible to inoculations with living cultures, and that in these animals a condition more nearly resembling human cholera is produced. The injections are successful when made into a vein (Thomas), or when, after neutralization of the gastric contents, the cultures are carried into the stomach (Issaëff and Kolle). According to these two authors young rabbits are most susceptible. Diarrhœa sets in, and the clinical picture is that of the algid stage of cholera. The appearances of the intestines are similar to those of human cases, and the distribution of the bacilli is identical. Still other animals are more or less susceptible. Newly born cats (Wiener) and young dogs (Karlinski) are affected by the ingestion of cultures. House-mice sometimes react, although field-mice and white mice are refractory. Pigeons are resistant, large doses being necessary in order to cause their death—a different reaction to that encountered with V. metchnikovii.

Method of Demonstrating Cholera Bacilli.—In cases of suspected cholera the fluid dejections are first examined microscopically. The slimy particles, consisting chiefly of swollen epithelium, are selected. These are stained in the usual way and examined. In some cases an immediate and positive diagnosis can be made in this way. Linen soiled with cholera dejections when kept for some time in a moist condition affords, as Koch has shown, very favorable material upon which to base a diagnosis, since under these circumstances the bacilli multiply rapidly. Sometimes the other intestinal bacteria have multiplied to such an extent that even should spirilla have been present they would be overgrown. Hence, in addition to the microscopical examination, cultures should be made. For this purpose it is also best to select some of the slimy particles contained in the dejections, although linen soiled with movements may also be employed. This material is suspended immediately in liquefied nutrient gelatin, and dilutions are made, after which Petri plates are poured. The charac-

teristic appearances of the cholera colonies serve to distinguish them from other intestinal bacterial growths. When the number of vibrios is small, this procedure may fail to reveal them; in such case the enriching method of Schottelius gives better results. A small quantity (one to several drops) of the suspected material is introduced into a sterile solution containing one per cent. peptone and one-half per cent. salt. The tube is incubated over night. The spirilla of Asiatic cholera, which are strongly aërobic and actively motile, seek the surface of the medium. In favorable cases a membrane may be formed at the end of six hours, consisting of almost pure cultures of vibrios. From this membrane or from the surface of the culture microscopical preparations and plate cultures are made. In examining surface waters, a concentrated solution of peptone and salt is added to 50 to 100 c.c. of the water, so as to bring the entire volume up to the strength of peptone-water. This is incubated, and cultures are made from the surface growths. The demonstration of the microorganisms in the intestinal wall is comparatively easy, provided that fresh cases are examined and sections are stained in methylene blue. In cases which have lasted a longer time, the organisms are much more difficult of demonstration in sections.

Cholera Poison.—The conception at first held by Koch, that cholera is chiefly an intoxication, the poison being absorbed from the intestine, and that the general symptoms are the result of this absorption, has led to a search for poisons connected with the organisms. Some have believed that the poison is secreted by the vibrios, others that it is a constituent of the bacterial cell. Ransom, Metchnikoff, Roux, and others believe they have shown that a soluble poison is formed. Hueppe believed that he had brought proof that more poison is formed under anaërobic conditions, and believed that for this reason the cultivation in eggs increased the toxicity. It appears not improbable that Hueppe's and Scholl's experiments were not free from contaminations, and Westbrook has shown that the aërobic cultures are more virulent than those grown under anaërobic conditions. R. Pfeiffer has found that the poison of the cholera organisms adheres to the bacterial cell and is still active even after the cultures have been carefully destroyed. Ten milligrams of a twenty-hour-old culture, which has been killed by being exposed for ten minutes to the influence of chloroform vapor, is sufficient, when injected intraperitoneally, to kill a guinea-pig weighing 200 gm. Intoxication first appears at the end of two or three hours. The animals usually succumb in from eight to twelve hours with symptoms of collapse, analogous to those of the algid stage of human cholera. The poison is easily injured by heating to 60° C., long drying, etc. In its action it

shows distinct differences from the bacterial poisons furnished by B. diphtheriæ, B. tetani, and other organisms, inasmuch as no period of incubation is needed. The rapidity of action will depend upon the rapidity of the absorption, which takes place most promptly when the poison is introduced into the circulating blood and less rapidly when it is injected subcutaneously. When carried into the intestine the poison is not absorbed so long as the epithelium is uninjured. On the other hand, injury to this structure is followed by fatal intoxication.

Immunity.—Human beings who have recovered from cholera contain in their blood a body which will protect guinea-pigs from the results of intraperitoneal inoculations of the cholera vibrios (Lazarus). Lazarus believed that this action was similar to that observed in the case of the antitoxins of diphtheria and tetanus. It was later shown by R. Pfeiffer that the injection of human serum caused rapid disintegration of the vibrios introduced into the peritoneal cavity of the animal, and thus prevented a fatal multiplication of the organisms. The principle upon which this action depends appears in human beings between the eighth and the tenth day of the disease, and may have entirely disappeared at the end of two or three months. Guinea-pigs, rabbits, and goats develop a similar substance in their blood after treatment by subcutaneous or intraperitoneal inoculations of dead cholera cultures. R. Pfeiffer has shown that when living cultures admixed with a small quantity of the serum of immunized animals are introduced into the peritoneal cavity of the guinea-pig, rapid disintegration of the organisms takes place, and moreover this action is specific for the cholera germ. Other vibrios are not affected. The agglutination reaction is also obtainable with this serum. Haffkine has prepared a vaccine which he has employed on a large scale in India. It consists of two concentrations (*premier vaccin anticholérique*, and *second vaccin anticholérique*). These are injected subcutaneously. The reaction is both local and general; the first consists of pain and moderate swelling of the skin, at the site of injection and in the neighboring lymph glands; the latter manifesting itself by a rise of temperature and slight indisposition. The injections are made six days apart. The second injection produces fever, but no local reaction.

Vibrio Massauah (Pfeiffer).—Obtained by Pasquale from the dejections of a man in Massowah, supposed to be suffering from cholera. With our present knowledge it is doubtful whether the localized epidemic which occurred at that place was real cholera. The organism, which for some years was regarded as identical with V. choleræ, shows morphological and pathogenic properties at variance with those of

the latter organism, and fails to give the Pfeiffer reaction with cholera serum. The chief morphological difference consists in the possession of four flagella. The colonies upon gelatin are also different, and liquefaction is much slower. Pathogenicity is more pronounced, approaching that of V. metchnikovii. The organism would appear sometimes to be the cause of infectious diseases in human beings, and its ingestion gives rise to cholera-like symptoms (Metchnikoff).

Vibrio Metchnikovii. *Synonym.*—Microspira metchnikovii (Migula). Obtained by Gamaleïa in Odessa, in 1887, from an epidemic among fowls. Pfuhl found it in 1893 in the Spree basin, and Kutchers in the Lahn. The symptoms in infected fowls are suggestive of chicken cholera; the vibrios are found in the intestine, and usually also in the blood. The morphology is similar to that of V. choleræ asiaticæ. The two organisms cannot be distinguished under the microscope. The more pronounced bendings and the usually greater thickness and smaller length of V. metchnikovii afford points of distinction. It is actively motile, and possesses a single polar flagellum. Gelatin is liquefied, as a rule, more rapidly than by V. choleræ, although specimens vary widely in this respect. The colonies in gelatin have a yellowish or yellowish-brown granular appearance, the edges being striated, with fine, hair-like radii sometimes going off from them. The gelatin stab, when the liquefaction is rapid, has a wider surface than is usual with the cholera spirillum. Bouillon is clouded, and a membrane formed. The nitroso-indol reaction is given without the addition of nitrites. Sugar is fermented, lævolactic acid being formed, but no gas is produced. One of the most striking points in which it differs from the cholera spirillum lies in its pathogenicity for pigeons and young hens. The introduction of a minute quantity of the culture into the muscles of the breast is followed by local and general symptoms resembling those produced by the bacillus of chicken cholera, the localized œdematous area and the blood containing enormous numbers of vibrios. The intestine is usually pale; it contains much fluid of a yellowish-gray color, in which desquamated epithelium is found in considerable amount, but only very few vibrios. Guinea-pigs are also susceptible, and usually succumb in twenty-four hours to subcutaneous injections. The local, spreading, bloody œdema contains large numbers of microorganisms, as does also the heart's blood. Infection can follow ingestion of cultures if the same precautions are taken as with V. choleræ. Large numbers of the spirilla are found in the intestinal contents, the blood, and the various organs. Although the pathogenicity of the cholera vibrio can be increased by passage from animal to animal, the grade

always remains far below that of V. metchnikovii. This divergence serves to distinguish the two forms, but the distinction can be made more certainly by means of the Pfeiffer reaction.

Spirillum Finkler and Prior. *Synonym.*—Vibrio proteus. Obtained by Finkler and Prior, in 1884, from the dejections of a case of cholera nostras, which had been collected for some time. The curved bacilli are longer and thicker than comma bacilli. They average from 0.4 to 0.6 μ in width and 2.4 μ in length. The gelatin colonies are finely granular and yellow with smooth edges. Liquefaction is rapid in the stab; it is tubular in form and the gas bubble characteristic of V. choleræ does not appear. Upon agar growth is more rapid than in the case of V. choleræ. The colonies resemble, under a magnification of 60° C., those of B. coli communis. Milk is coagulated, the clot being after a time peptonized. A small amount of acid, but no gas, is formed out of glucose. The indol reaction is slight, often failing altogether. The organism has since been occasionally found in the dejections of healthy persons as well as in a few cases of diarrhœa and of suspected Asiatic cholera. It probably has no relation to this disease, or even to cholera nostras. Its pathogenicity for animals is less marked than that of V. choleræ.

Spirillum Tyrogenum. *Synonyms.*—Deneke's cheese spirillum; Vibrio tyrogenes (Lehmann and Neumann); S. terrigenum (Migula). Isolated by Deneke (1885) from an old cheese, since which time it has been rarely found. The curved bacilli are smaller than cholera vibrios and often tend to appear in the form of long spirals. With regard to liquefaction the organisms stands midway between V. choleræ and V. Finkler-Prior. By Pfeiffer the indol reaction is said to be absent, but Lehmann and Neumann state that the organism with which they worked produced it to about the same extent as V. choleræ. Growth does not take place upon potato either at the room temperature or in the thermostat.

Cholera-Like Water Vibrios.—During the last epidemic of cholera in Europe great activity was displayed in searching for spirilla in human environments, especially through the use of the enriching method of Schottelius, and a very considerable number of vibrios were thus discovered. The number is now so great, and the classification so defective, that the subject cannot be handled with profit in this place. The great interest in all concerns their relationship to the vibrio of Asiatic cholera. Many of the forms are pathogenic, and in morphology and cultural properties stand near the well-known virulent forms. Frequently the serum reaction of Pfeiffer is needed in order to distinguish them from the organisms associated with definite diseases, with which they might be confounded. In this country

Abbott has obtained a pathogenic spirillum (V. schuylkilliensis) from the Schuylkill River at Philadelphia, which in cultural and pathogenic properties is closely related to V. metchnikovii, although it may be distinguished from it by the serum reaction.

Spirillum Obermeieri. *Synonym.*—Spirochæte Obermeieri (F. Cohn). Large, flexible, motile, corkscrew-like threads with sharpened ends. The organism occurs in the blood and in the spleen of human beings suffering from recurrent fever. It was discovered by Obermeier in 1873. The individual spirilla are from one and a half

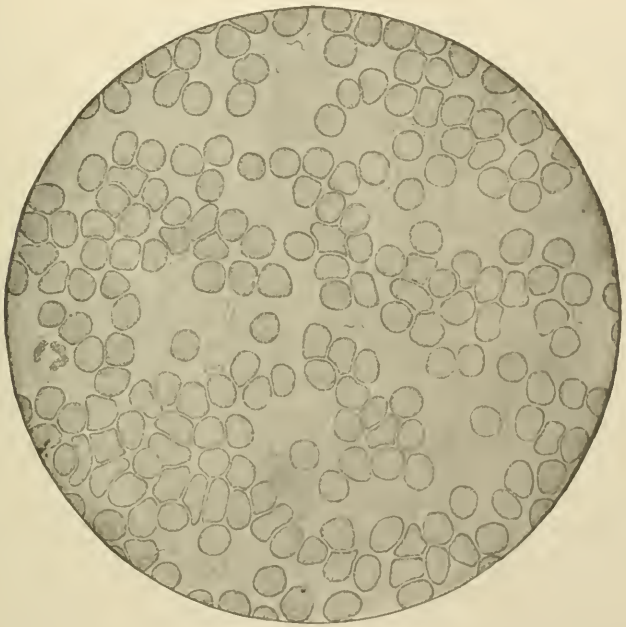

Fig. 33.—Spirillum Obermeieri in the Blood from a Patient with Relapsing Fever. From a dried specimen. × 560. (Popoff.)

to twenty-six times as large as a blood corpuscle. They are usually found during the height of the fever, and once were demonstrated by Naunyn in the afebrile period. Karlinski has shown that the organism is connected with some cases of febrile icterus. The spirilla begin to appear in the blood shortly before the appearance of the elevation of temperature. They increase during the fever, and then a short time before its cessation begin to disappear. They are to be regarded as pronounced blood parasites. In one case they have been found in the urine, but in this case blood was also present. They are motile, possess flagella, and move, by rapid twistings of their bodies, first to one and then to the other side of the microscopic field.

Their presence may be recognized even with a low power of the microscope owing to the commotion which is caused among the blood corpuscles. Staining can be easily accomplished by ordinary watery anilin dyes, but not by Gram. Cultivation experiments thus far have given negative results. In the afebrile period bodies of peculiar refraction and suggesting diplococci have been described. They have been regarded by some as spores of the organism which at that period is present in the internal organs, especially in the spleen (Sarnow, Jaksch). From the fact that these bodies were found also in other diseases, they were thought to be without significance. Albrecht, however, collected blood during the apyretic stage and preserved it in a moist chamber. He claims by this procedure to have cultivated the spirilla from the above-mentioned bodies. Inoculation experiments are successful, from man to man (Mocutkowski), and from man to monkey (Koch, Carter, Metchnikoff). In the latter a single attack of fever only is usually produced. According to Metschnikoff, after the pyretic stage, the spirilla again accumulate in the spleen of the monkey, and are taken up by phagocytes. They also appear in the form of thick balls. Soudakewitch removed the spleen from monkeys, and then inoculated them with blood containing the organisms; all the animals died. His conclusion, that splenectomized animals are a favorable medium for the unrestricted development of the spirilla, has been controverted by the control studies of Tictin, who obtained totally different results. Nikikoroff studied the spleen in fatal cases of recurrent fever. He demonstrated the organisms in this situation by the use of a methylene-blue solution containing decolorizing substances such as tropæolin, or fluorescin. He describes numerous infarctions, which seem to be associated with the development of the microorganism. The staining-agent is prepared as follows:

One-per-cent. alcoholic tropæolin solution, . . .	5 parts.
Conc. aqueous methylene blue, ...	10 "
Water, ...	10 "

To this solution at the time of its employment may be added from two to five drops of a 1:1,000 solution of caustic alkali. For staining, several hours are required.

Anaerobes.

These bacilli have two features in common. They grow only in the absence of oxygen; and their colony formations are usually associated with the development of hair-like processes, which give to the growth a thorn-apple appearance. They are sporogenous, the dis-

tinctions between the various kinds and groups in part depending upon the mode of formation of the spores. Other differences, especially in pathogenicity, are also of importance. Gelatin is usually liquefied and at the same time decomposed with the production of foul-smelling products.

Group of Malignant Œdema Bacillus.

Bacillus of Malignant Œdema. *Synonym.*—Vibrion septique (Pasteur).

Morphology and Cultural Properties.—The organisms appear as large rods, which, more particularly in cadavers, tend to grow into long threads. Some varieties show an active motility produced by peritrichal flagella (eight to twelve), which, however, are usually found only on the short forms. The organisms vary from 0.8 to 1 μ in thickness, and from 2 to 10 μ in length. The edges are rounded. Spores are formed, occupying the mid-portion or lying near the centres of rods, which here show very slight thickening. Sometimes, however, the spores attain an unusual thickness, and cause a swelling of the organism. Staining takes place with ordinary dyes, and decolorization follows the use of Gram's method, unless immersion in the stain has been prolonged (twenty-four hours at 37° C.) and a mordant, such as anilin oil or carbolic acid, has been employed. Double staining of spores and vegetative forms succeeds. In cultures the organism is difficult to distinguish morphologically from the organism of symptomatic anthrax (page 743). For cultivation an absence of oxygen is imperative. In gelatin the colonies show the thorn-apple appearance, while in agar this feature becomes even more strikingly marked, the colonies being made up of a thick network of threads. In stab gelatin the growth is more continuous, appearing as a white line with short side processes; gas bubbles are formed, and the medium is liquefied. Eventually the growth settles to the bottom. Bouillon is decomposed, gas is liberated, and the medium is rendered turbid. Litmus is reduced, and the casein of milk is coagulated. Growth takes place upon potato, and according to Kerry and S. Fraenkel, carbohydrates are split up with separation of butyric acid. In sugar media ethylic alcohol and lactic acid are formed (Kerry). The organism is widely distributed in nature, occurring in earth, stagnant water, hay infusions, and elsewhere. Pasteur obtained it by in-

Fig. 34.—Bacillus Œdematis Maligni. From an agar culture, showing spores. × 1,000. (Fränkel and Pfeiffer.)

jecting putrefying fluids into animals. R. Koch was the first to give an accurate description of the microorganism, and called it by the name which it now bears, although Pasteur had pointed out its anaërobic nature. It was obtained in pure cultures first by Liborius.

Pathogenicity.—The organism may be isolated by inoculating certain suspected materials subcutaneously into guinea-pigs. At the site of the puncture there forms a hemorrhagic œdema which may extend over a large part of the subcutaneous tissue of the animal. Immediately after death the bacilli are confined to the local œdema, but after some hours, up to a day or two, they are also found in the internal organs, being more numerous and more widely distributed the longer the time which has elapsed since the death of the animal. Mice and rabbits are also susceptible, although pure cultures of the organism vary greatly in their activity. According to some observers, and with certain specimens, a few drops are sufficient to produce the characteristic lesions, whereas at other times, and in the hands of other investigators, several cubic centimetres are required. The simultaneous inoculation of other organisms, though themselves non-pathogenic (B. prodigiosus, B. proteus), greatly increases the virulence of B. œdematis maligni. The part played by this organism in human pathology is as yet undetermined. The literature contains numerous references to its occurrence in gangrenous emphysema, of which it was believed to be the cause. At an earlier period, when certain mystical influences were supposed to reside in various kinds of unclean substances with which wounds were treated, an œdema, presumably caused by this organism, was not an unusual occurrence. It is now thought, however, that a considerable proportion of the gaseous phlegmons, formerly attributed to the action of this organism, are actually caused by another anaërobic species, B. aërogenes capsulatus (page 737). Brieger and Ehrlich describe the first cases of supposed infectious malignant œdema in human beings. Two patients suffering from typhoid fever received injections of tincture of musk. Death occurred three days later; at the sites of the injections there was a marked œdema, from which were isolated organisms which culturally and in pathogenicity agreed with the bacillus of malignant œdema. König and Rosenbach have described suspicious cases, and Brunner has detailed a case of emphysematous gangrene following abortion in which œdema and crepitation were found in the pectoral region. The patient died and an organism, supposed to be B. œdematis maligni, was obtained from the local lesion and the internal organs. Witte believed that he isolated possibly this organism in a case of pyosalpinx, while Hlava, in 1891, possibly obtained it from a case of compound fracture of the radius, fracture of ribs, and other injuries

in which emphysema of the subcutaneous tissues in several parts of the body had appeared. Other pathogenic organisms may find their way into wounds at the same time. Thus Labit reports a case of compound fracture in which the wound was contaminated with earth. On the third day, malignant œdema, and on the fourth day, tetanus appeared. Giglio has observed in a periuterine abscess the staphylococcus aureus and the bacillus of malignant œdema. Both organisms are supposed to have emigrated from the rectum. The bacillus of malignant œdema produces an active poison, which when injected into a guinea-pig causes the death of the animal. Through the use of this toxin immunization can be brought about. The bouillon cultures, filtered or heated, are employed for the injections, or the œdematous fluid is used in quantities less than are needed to cause fatal results. Animals can be protected against the living bacilli, but not against the organisms of symptomatic anthrax and tetanus (Roux, Sanfelice).

Bacillus Pseudoœdematis.—This organism was found by Liborius in infected samples of earth often associated with the bacillus of true malignant œdema. The rods are somewhat thicker, and in the single thread several spores are formed, which do not extend beyond the surface of the bacilli. The colony formation and other cultural properties are very similar to those seen in the bacillus of true œdema. Its pathogenicity is slight or nil. Inasmuch as Sanfelice has found that guinea-pigs, by means of filtrates of this culture, can be immunized from the bacillus of malignant œdema, it is highly probable that this organism is merely a modified and weakened variety of the latter. Sanfelice also states that when cultivated in a medium containing the poison of B. tetani this organism acquires the virulence of the true œdema bacillus.

Bacillus Aerogenes Capsulatus. *Synonym.*—B. emphysematosus (E. Fraenkel). First described by Welch and Nuttall in 1891, the organism has since been found by a large number of bacteriologists in various parts of the world, to be associated with emphysematous gangrene, and the production of gas in the internal organs and in the blood. It invades living tissue, although it develops more readily and with the production of much gas in necrotic tissue, and in the body after death.

Morphology and Cultural Properties.—The bacillus is non-motile, straight, or sometimes slightly curved. In thickness it corresponds to the anthrax bacillus, and averages from 3 to 6 μ in length, with the adjacent ends slightly rounded or sometimes square cut. It grows singly, in pairs, in clumps, and sometimes in short chains; less frequently in threads and long chains; it stains readily with the ordi-

nary anilin dyes, including Gram's and Weigert's stains, either uniformly or showing small unstained spots; less frequently isolated deeply stained granules appear. Although the periphery does not stain, a capsule is frequently demonstrable in specimens from the animal body, and sometimes from agar cultures. Spores do not tend to form in the animal body, although in a few instances they are said to have been found in this situation; they develop only rarely in artificial cultures, excepting upon blood serum. The bacillus grows upon all ordinary culture media, rapidly at the body temperature, slowly at temperatures as low as 18 to 20° C. It is anaërobic, no growth occurring on the surface of solid media or in ordinary fluid cultures in test-tubes exposed to the air. In the depth of solid media exposed to the air growth can take place, while under anaërobic conditions it occurs both upon the surface and in the depth of solid media. Gas is produced in all cultures containing fermentable material. The colonies in agar present a grayish-white to a more opaque white or brownish-white color by transmitted light, sometimes with a central darker dot. At the end of twenty-four hours they average 0.5 to 1 mm., but they may subsequently attain a diameter of 2 to 3 mm. or even more. The surface presents irregular contours, the irregularity being due to knob-like or feathery projections. No putrescent odor is developed in cultures, unless a considerable amount of the agar culture is crushed with a little bouillon. In gelatin cultures there appears a limited liquefaction of the medium manifested by a settling of the growth, and slight displacement of gas bubbles. Bouillon is at first diffusely clouded, becoming clearer later as settlement of an abundant whitish precipitate takes place. Milk is coagulated in from twenty-four to forty-eight hours, the clot being firm and furrowed with marks of gas bubbles. Anaërobic potato cultures show either no visible growth, or a thin, moist grayish-white film on the surface, together with an abundant formation of gas in the fluid accumulated about the bottom and sides of the potato. In ascitic fluid there is abundant growth with formation of gas bubbles. The vitality of the cultures is very variable, depending upon the character of the medium and the mode of cultivation. Cultures in sugar bouillon, in an atmosphere of hydrogen, may be dead in three days or less. Similar cultures in Buchner jars may survive four months or longer. Cultures in sugar media are shorter-lived than those in plain media, and in tubes hermetically sealed after two or three days' growth the organism may survive several months. The thermal death-point is 58° C., in ten minutes.

Pathogenicity.—The organism was first isolated by Welch from the cadaver of a man who had suffered from tuberculosis and from an aneurism of the aorta which had perforated by a small opening

through the anterior thoracic wall, and which had given rise to repeated external hemorrhages, the last occurring two days before his death, which was sudden. No subcutaneous emphysema had been noticed during life. The autopsy was made in cool weather eight days after death. There was no evidence of post-mortem decomposition. Emphysema of the subcutaneous tissue was noted over the greater portion of the body. The blood-vessels of the heart contained numerous gas bubbles, and bubbles were abundant in the internal tissues, notably in the myocardium, liver, spleen, and kidneys, these organs presenting the appearances described by German writers as those of *Schaumorgane*. Gas bubbles were numerous in the clot which nearly filled the aneurysmal sac. The microscopical examination of the organs showed masses of bacilli in the diseased tissues, especially in the neighborhood of the gas blebs. The organism was obtained in cultures. About a year and a half later E. Fraenkel reported four cases of gaseous phlegmons. In one of these B. aërogenes capsulatus (the gas bacillus) was found unmixed with other bacteria, whereas in the remaining three it occurred in conjunction with other bacteria, chiefly pyogenic cocci. In the case of pure infection affecting the right lower extremity, there was no suppuration in any part of the enormous emphysematous swelling. On incision a turbid brownish fluid without offensive odor and containing gas bubbles escaped. The muscles were completely disintegrated. In two of the cases the gaseous phlegmon followed hypodermic injections of camphor oil and ether, and of a dilute solution in water of sulphuric acid and muriate of morphine respectively. Numerous publications have more recently appeared, indicating a wide distribution of the organism and its participation in a considerable number of pathological processes. In a great majority of cases the invasion occurs after death or during the death agony, development taking place immediately after death. There are, however, several instances now on record in which the organism had developed during life. Of these perhaps the most remarkable is that reported in 1893 by Graham, Steward, and Baldwin.

The patient was a laboring woman, married, aged 35. The symptoms came on about fourteen hours before death. The patient had previously been well. About ten hours after the first indication of illness she was found to be emphysematous over her entire body. This condition increased very rapidly after death, so that at the end of eighteen hours the body was double its normal size. The autopsy showed a recent abortion, the uterus containing bloody fluid and placental débris, which had probably afforded a portal of entrance for the microorganisms.

P. Ernst reports two cases in which the organism has been found;

in one the invasion followed the removal of a macerated four-months fœtus, in the other it occurred after a laparotomy for hernia. The number of cases of surgical infections is now considerable (Welch and Flexner[35]), instances having been reported by many writers in this country (Welch and Flexner, Mann, Dunham, Howard, Williams, and others). The organism has been shown to invade the internal organs by a number of paths, as, for example, the genito-urinary tract (Goebel, Williams, Welch and Flexner), the intestine (Welch and Flexner, Howard), the lungs (Welch and Flexner), and the biliary passages (Howard). Its presence is not uncommonly associated with peritonitis, especially the perforative variety. It may exist alone in the peritoneal cavity, although it is more commonly found associated with the streptococcus and the colon bacillus. It is capable of penetrating into the abdominal cavity without the occurrence of actual perforation of the intestine. It has been known to produce pneumoperitoneum during the life of the patient. One such instance was reported by Welch and Flexner, and another was observed by the writer in Manila in a soldier who succumbed to a strangulated mesenteric hernia, and in whose greatly distended abdominal cavity a pure culture of the gas bacillus was found. The organism also occurs in the lower animals, having been isolated once from a peritonitis in a rabbit following perforation of a round ulcer of the stomach (Welch and Flexner). The lower animals are susceptible to inoculation. Rabbits do not succumb after intravenous inoculations unless, as it would seem, dead tissue is present upon which development may take place. One such instance of successful intravenous inoculation in a pregnant rabbit was reported by Welch and Nuttall. Two of the embryos were macerated, dark, partly destroyed, and smaller than the others, which were intact. It is considered probable that these embryos in the uterus were already dead when the injection was made, and that as a consequence the bacilli were able to gain a foothold and develop. Subcutaneous inoculations in the rabbit may be entirely without effect, or may produce localized abscess from which the animals usually recover. Guinea-pigs and pigeons are more susceptible. Subcutaneous inoculation, in the case of the guinea-pig, causes a process essentially identical with the condition observed in human patients. Gas appears usually within the first twenty-four hours, and death may occur as early as the end of the first day or as late as thirty-six or forty-eight hours after the injection. In some cases the skin over the infiltrated area bursts, and after prolonged necrosis and ulceration recovery by cicatrization may occur. At autopsy the muscles and the subcutaneous tissues are found to be converted into a soft pulpy mass containing blood-stained fluid and

gas bubbles in large number. There may be extension of inflammation to the peritoneal and pleural membranes. In pigeons the process is more marked and more rapid. Injections of fluids containing many bacilli into the pectoral muscles may cause death within seven hours after the inoculation. Death usually occurs at the end of twenty-four hours, with the presence of gaseous œdema and necrosis of the muscle. The bacilli are extremely numerous in the necrotic tissue. In rare instances after necrosis and ulceration the animals recover. Mice may be killed by subcutaneous inoculation.

It seems probable, as was originally pointed out by Welch and Nuttall, that the greater number of instances reported in the earlier literature of the suspected entrance of air into the blood-vessels immediately before death, or in which gas was found in the tissues at fresh autopsies, were probably due to the development of this bacillus, or some other gas-producing microorganism. These cases have been noted more particularly after criminal abortions; and the number of the instances of uterine infection with this organism now on record would seem to bear out this view. Moreover, it is highly probable, as Welch and Flexner have pointed out, that in the cases reported before 1892 under the names of malignant œdema, emphysematous or gas ous gangrene, gaseous phlegmon, emphysematous cellulitis, gangrenous septicæmia, gangrène gazeuse foudroyante, etc., the bacilli usually described as those of malignant œdema were in some instances identical with B. aërogenes capsulatus.

Bacillus Botulinus.—Described by Van Ermengem in 1897 as the cause of an epidemic of meat-poisoning (botulismus).

Morphology and Cultural Properties.—An anaërobic bacillus averaging from 0.9 to 1.2 μ in width and from 4 to 9 μ in length. It is a straight rod with slightly rounded ends resembling B. anthracis and B. œdematis maligni. The rods usually occur singly, and only occasionally in pairs or in short threads. Spindle-shaped forms are common.

Agar-agar and gelatin cultures.—In older cultures involution forms appear. Spore formation takes place under some conditions. The spore forms near the end, more rarely in the middle portion of the rod; it is oval and somewhat thicker than the rod itself. The spore formation is influenced by temperature, and by the composition and reaction of the medium. It is most common in gelatin cultures that are strongly alkaline and contain two per cent. glucose. At temperatures above 35° C. sporulation rarely takes place. The bacillus is highly motile, and is provided with four to six undulating flagella. It stains by Gram's method, if the alcohol application is not too long continued. Young colonies in glucose gelatin are characteristic. At

the end of from four to six days, and under a magnification of forty to sixty diameters, they are spherical, translucent, of a pale yellowish-brown color, and composed of fairly coarse, highly refractive granules, which seem to be in continuous motion in the periphery of the colonies. Surrounding the colony is a thin zone of liquefaction. At a later date the colonies become opaque, although the motile granules may still be made out in the outer portions. The periphery is now occupied by groups of short rods; at a still later period it becomes very irregular, and presents an incised appearance, with long processes growing into the incisions. Stab cultures in sugar-gelatin develop in the form of small, white, round masses, and in the course of the stab, if the gelatin is soft, rays penetrate in all directions, the medium becoming liquefied about the growth. Gas development takes place, breaking up the gelatin, until the entire mass may eventually become fluidified, the growth sinking to the bottom. The agar-agar cultures are not characteristic. The odor from cultures is not especially disagreeable even after a long time, but is somewhat suggestive of butyric acid. Saccharose and lactose are usually not fermented. Growth is said not to take place upon potato. Glucose bouillon is uniformly clouded and large quantities of gas are generated. Milk is unaffected.

Pathogenicity.—The organism was first obtained, in 1895, from cases of meat-poisoning occurring in a small town in Belgium. With few exceptions, all who partook of the spoiled meat became ill. Three died; some barely escaped with their lives. The poisoning was traced to raw ham. Symptoms came on for the most part in from twenty to twenty-four hours, and in some cases thirty-six hours after the meal. They consisted of pains in the stomach, repeated vomiting and diarrhœa, which was followed by obstinate constipation. The vision was obscured so that even near objects could not be distinguished, and there was binocular diplopia. The organism was cultivated from the spleen of one of the fatal cases, and was also obtained from a portion of the suspected ham. The ingestion of the infected meat caused death in mice. Rats and cats did not die as a result of the feeding experiments. Guinea-pigs succumb to the ingestion of macerations of the poisonous material. Subcutaneous inoculations are successful in mice, pigeons, monkeys, and cats, and in rats if somewhat large doses are employed. Dogs and chickens are refractory. In cats and monkeys symptoms have occurred suggestive of mydriasis. Rabbits succumb to small quantities of the macerations in from six to ten or twelve hours. After an incubation period of six to ten hours the symptoms come on suddenly; the animal becoming paralyzed, and developing convulsions in which death takes place. The

study of filtered macerations, made from the original infected ham, proved that the morbid effects depended upon the existence of a soluble toxic substance. Experiments have shown the poison to be of extreme potency. Fifty milligrams of the solid constituents of the maceration is sufficient to kill quickly rabbits of the combined weight of 100,000 kgm. As applied to human beings, of an average weight of 70 kgm., this would represent sufficient poison, if injected subcutaneously, to cause the death of fifteen hundred persons; hence the minimal lethal dose for an adult human being may be estimated at 0.035 mgm. The intensity of the poison becomes evident when it is compared with the lethal dose (for human beings) of the tetanus toxin; according to Brieger, the latter substance in a moderately pure state proves fatal in 0.13 mgm. doses. Further experiments have shown that in the living animal after introduction of B. botulinus no fresh toxin is formed, the pathological effects being due entirely to a pre-formed poison. This poison is injured by high temperature. At 70° C. its virulence is almost completely destroyed in one hour; at 80° C. in a half hour, while three-hours' heating at 58° C. reduces its activity without completely destroying the poison. At 100° C. the action is much more rapid. Infected flesh, therefore, exposed to the boiling temperature, and kept there for a certain period, would be rendered innocuous. Drying does not destroy the poison. It is very slowly dialyzable, and putrefaction does not injure it. Its chemical nature, while thus far not clearly established, corresponds, so far as our present investigations go, with the toxalbumins of diphtheria, tetanus, etc. The growth of the microorganisms outside of the body in artificial cultures is associated with the production of poison, which, after separation from the bacilli, is capable, when introduced into animals, of causing the same group of symptoms as those produced by macerations obtained from the originally infected meat.

Group of Symptomatic Anthrax Bacillus.

The organisms included in this group differ from the preceding more particularly in respect to the manner of their sporulation. The spores are located either in the middle or near the end of the rods; in consequence of their greater diameter, their presence is marked by considerable swellings. The two main forms included by Kruse in this group are the bacillus of symptomatic anthrax (*Rauschbrand*) and the butyric acid bacillus. Only the first will be considered in this article.

Bacillus Anthracis Symptomatici. (*Synonyms.*—Rauschbrand Bacillus; B. chauvœi; Bact. sarcemphysematis (Kitt); B. du charbon

symptomatique. First discovered by Bollinger and Feser, and somewhat later by Arloing, Cornevin, and Thomas. First cultivated in solid media by Kitasato.

Morphology and Cultural Properties.—The bacilli are more slender than those of malignant œdema. They usually occur isolated, and never appear in such long threads as are attained by B. œdematis maligni in the animal body. Their length is from 3 to 5 μ. The ends are somewhat rounded. There is active motility due to peritrichal flagella. They stain by Gram. The spores are short and ellipsoidal, and occupy either the middle or the end of the rods. Involution forms are common both in cultures and in the animal body. According to Kitasato sporulation in the animal body takes place only after death. In infected meat when dried the spores remain alive for very long periods. The organism may be cultivated in the absence of air in fluid cultures; for this purpose a bouillon made of chicken and containing glycerin is especially recommended. Colonies in gelatin resemble those of B. œdematis maligni but develop gas more readily. Those in agar-agar are more compact, and numerous side branches are developed. Gelatin is liquefied. Litmus-milk is decolorized and coagulated. Starch is converted into sugar. Growth takes place between 16° and 18° C.; according to Kitasato, sporulation is most abundant at 37° C.

Pathogenicity.—The organism is the cause of symptomatic anthrax, a serious and often fatal disease affecting cattle, which was formerly confounded with anthrax. The bacilli are found in the sanguinolent œdema, in the muscles, and intestinal contents and bile of affected animals. The disease begins with the appearance of emphysematous gangrene usually of the extremities. The lymph glands in the neighborhood become swollen, fever appears, and then stupor, the animal succumbing in from twelve hours to three days. Hemorrhagic exudates into the serous cavities, sometimes peritonitis, and enlarged spleen are found. Infections, although taking place most commonly through the skin, may also penetrate through the mucous membranes. Young cattle (one to three years), goats, sheep, and especially guinea-pigs, are susceptible to experimental inoculation. Human beings seem to be immune, as do mice, rabbits, rats, swine, dogs, and cats. Horses react only locally to inoculations. Sanfelice has pointed out that with pure cultures large quantities (up to 4 c.c.) are necessary to kill guinea-pigs if injected subcutaneously. Kitasato possessed more virulent cultures in which the dose was from 0.1 to 1 c.c. Immediately after death the organism was found only locally, but as in the case of malignant œdema, at a later period it could be demonstrated in the internal organs and even in the blood. On the other hand infusions

of infected meat, containing other organisms in addition to this bacillus, are much more active. Soluble poisons are formed so that the filtered cultures are toxic. Inoculation with infected meat which has been dried and heated for some hours to 100° C. sometimes gives immunity to the natural disease. Similar effects have been obtained by injecting minute quantities of the active virus, or larger quantities of cultures which have been weakened through the use of chemicals.

A slightly different organism (Pseudo-Rauschbrand bacillus) has been obtained by Sanfelice from putrefying flesh infusions and earth. It resembles the true bacillus but lacks its pathogenic properties. According to Sanfelice, when the organism is cultivated in a medium containing the tetanus poison it assumes virulent characters.

Group of Tetanus Bacillus.

This group includes the bacilli developing spores at their extremities, so that the latter present a definite swelling. The spores are spherical or nearly so.

Bacillus Tetani.—First observed by Nicolaier, who inoculated garden earth into various animals. Kitasato was the first to cultivate

Fig. 35.—Bacillus Tetani. × 1,000. (Lehmann and Neumann.)

in a pure state the organism, previously seen and described by Nicolaier.

Morphology and Cultural Properties.—Rods varying from 1.2 to 3.6 μ in length and 0.5 to 0.8 μ in breadth. Long threads are common; sometimes the rods remain united so as to form chains. The spores, which occur at the ends of the rods, are usually round or slightly elongated. They average from 1.5 to 2 μ in length. Spores lying side by side are sometimes present in the long threads (Lehmann and Neumann). Motility is very slight, or may be entirely wanting notwithstanding the possession of numerous long peritrichal flagella. Staining by Gram is positive. Parasitic forms of the organism obtained from human beings or animals are absolutely anaë-

robic. A prolonged saprophytic existence, as in artificial cultures, renders the organism somewhat less sensitive to oxygen, while in the presence of saprophytes the organism grows in an oxygen-containing atmosphere. The growth is active between 36° and 38° C. and fails at 14° C. Colonies in gelatin are first small, white, and punctiform, and soon become surrounded with a zone of liquefaction. Under moderate magnification they are yellowish-brown in color, and small hair-like processes are seen extending into the surrounding medium. These are thickly interwoven and frequently corkscrew-like. The older colonies show longer and more irregular processes, which eventually become granular and disintegrate. Gelatin stabs show at first a cloudy growth; as bubbles develop, liquefaction sets in, and a granular deposit is formed. The side processes consisting of fine twisted hairs penetrate at right angles into the surrounding medium. The characteristic felted appearance seen in gelatin colonies is also evident in agar-agar. In this medium liquefaction does not take place. Blood serum is sometimes fluidified, at other times not. Cultures in bouillon show a moderate clouding. Milk is not coagulated. Very slight growth takes place in sugar-free media, and also in Uschinsky's fluid. Sugar is fermented without the production of acid (Lehmann and Neumann). In the absence of sugar gas is said not to form. H_2S is produced in large amount, but no indol. According to Tizzoni and Cattani, cultures of diminished pathogenicity produce acid when grown upon sugar media. At a temperature of 37° C. sporulation can be noted at the end of thirty hours.

FIG. 36.—Bacillus Tetani. Culture in gelatin. (Kitasato.)

The microorganism is extremely widely distributed. It is present in earth, especially in gardens or about stables where horses are kept and also in the dust of hay. The inoculation of material from those sources into animals commonly produces tetanus. The bacillus has been found in the dejections of horses and cattle.

Pathogenicity.—In human beings the tetanus bacillus is the cause of trismus and the various forms of tetanus—traumatic, puerperal, and of the new-born. The organism is found in small numbers in the secretions of infected wounds; it does not appear in the internal organs nor in the blood. The disease appears as a spontaneous infection in human beings and among domestic animals, commonly in

horses, more rarely in sheep, goats, and other domestic animals. Carbone and Perrero obtained some of the bronchial secretion of a man affected with so-called rheumatic tetanus, and injected it into animals. These inoculations produced tetanus, and a supposed tetanus bacillus was obtained which, however, grew aërobically but with a loss of virulency. Kruse also isolated from a case of traumatic tetanus an aërobic organism that was no longer capable of producing tetanic symptoms. The bacilli of tetanus are introduced into the body through wounds which in some cases are very small or which may have entirely healed before the outbreak of the tetanic symptoms. Frequently the infectious material contains other microorganisms in addition to the tetanus bacillus. It has, however, been shown that pure cultures of tetanus bacilli produce in animals the classical symptoms of the disease. With the exception of the so-called rheumatic varieties, about the origin of which there still remains some obscurity, all the forms of tetanus have now been definitely shown to be due to the invasion of this specific bacillus. There are no grounds for believing that the poison enters the body except through the skin surface. It is probable that in the obscure rheumatic forms the break in the skin through which the material has been absorbed has been sufficiently small to escape notice, or else there has been some slight wound which has entirely healed. The microorganisms, having once gained access, multiply in the wound. For the production of the clinical picture of tetanus the multiplication need not be very great, since the potency of the generated poison is intense. The poison is absorbed from a traumatic focus into the blood current, and exerts its chief pathological action upon the spinal cord. It is demonstrable in the blood, as has been shown by Stern, and is eliminated with the urine, as has been proven by Bruschettini. The development of tetanus after wounds have been treated with earth, spiders' webs, and other extraneous materials depends upon the presence in these substances of the spores of the tetanus bacillus.

Mice, rats, guinea-pigs, and rabbits are susceptible to the action of pure cultures or of filtrates. The incubation period varies from one to three days. The amount required will depend somewhat upon the age of the culture employed. Thus in old cultures very minute quantities—the amount that would adhere to a platinum needle—is sufficient to kill mice and guinea-pigs. Other animals are less susceptible, pigeons being far more refractory, and chickens almost insusceptible. Very small doses produce a subacute or chronic form of tetanus which, after lasting for days or weeks, may be followed by recovery. The lesions in the internal organs, both of human beings and of animals who have succumbed to tetanus, are very trifling.

Toxin.—Filtrates of tetanus cultures when injected into animals produce the same effects as the living germs, except that in the former case the symptoms of intoxication appear more quickly. The first visible effect is seen in the muscle groups in the neighborhood of the site of the injection. Thence additional, successive muscle groups are involved, the poison appearing to travel along the course of the nerves. The poison is present not only in the blood, but also in the internal organs, such as the liver, spleen, and kidneys, so that inoculation with macerated portions of tissues from these sources produces the symptoms of intoxication. The amount of poison which is manufactured varies according to the source and age of the culture, as well as with the composition and reaction of the media. Long keeping and access of air, as well as the influence of various chemicals, and temperatures of 55° to 60° C. destroy its virulence, which is much more persistent in the dried substance. The poison belongs to the group of toxalbumins. Its potency is almost incredible. According to Vaillard and Vincent the lethal dose for a guinea-pig is 0.000025 gm., and for a mouse 0.00000025 gm.

Immunization.—Behring and Kitasato have shown that animals may be immunized to tetanus by rendering them insusceptible to the poison produced by the specific bacillus. The methods which are now employed in most countries consist in injecting into large animals, the goat or the horse, filtrates from tetanus cultures which have been treated with certain chemicals (trichloride of iodine, iodo-iodide of potassium) until they are weakened so that the animal reacts with only moderate symptoms. Successive injections are made of stronger and stronger poisons as the resistance is increased. Eventually the animals resist not only injections of the poison, but also inoculations with large quantities of cultures. The blood serum of animals treated in this way contains an antitoxic body capable of protecting others against infection, and even of curing them after the symptoms of intoxication have appeared. Marked success has attended the preventive treatment, failure being by no means excluded after the development of the tetanic symptoms. This method has been applied to the treatment of human beings in its latest form by injections made directly beneath the dura mater.

Method of Isolating the Tetanus Bacillus.—To obtain the organism from the secretions of wounds or from suspected soil is a matter of some difficulty. Occasionally the organism may be demonstrated in cover-glass preparations from the exudate; where such examinations have proved negative, cultural procedures are often of service. Owing to the fact that there is usually an admixture with other organisms the method of isolation is somewhat intricate and is as follows:

Mice are inoculated subcutaneously with infected earth. The secretions from the wounds of those animals which have succumbed to tetanus are spread upon the surface of blood serum or agar tubes, and incubated at 37° C. for twenty-four hours. Microscopic examination of the cultures will usually show the presence of bacillus tetani admixed with other organisms. The cultures are now subjected to a temperature of 80° C. in a water-bath for from forty-five minutes to an hour. After this Esmarch tubes or plates in slightly alkaline gelatin are made with small amounts of the cultures, and incubated in an atmosphere of hydrogen. At the end of several days the tetanus bacilli may be recognized by the form of the colonies, from which they may be isolated. In this method, the principles of which were introduced by Kitasato, advantage is taken of the fact that at 80° C. vegetative forms are destroyed, whereas the spore-bearing tetanus bacillus is still capable of multiplication. Since at this high temperature the organism is somewhat reduced in virulence, Lehmann and Neumann recommend a temperature of 60° to 65° C., which suffices to destroy the vegetative organisms.

Several PSEUDOTETANUS BACILLI have been described. These present the morphology of the bacillus of true tetanus, but differ from it in being non-pathogenic and non-toxic. One form described by Kruse grows aërobically, and is also non-pathogenic. Its relation to the organisms isolated from the bronchi in cases of rheumatic tetanus is still to be worked out.

Leptothricees, Cladothricees, and Streptothricees.

Under this heading will be considered those vegetable microorganisms the exact position and limits of which have not been defined. According to some authorities they not only bear a close relationship to the hyphomycetes but in reality represent imperfectly known specimens of this class. Lehmann and Neumann indeed go so far as to include the several bacilli which cause diphtheria, tuberculosis, and leprosy, under the special names of corynebacteria and myobacteria, with hyphomycetes. The groups of Cladothrix and Leptothrix are grouped under the special designation of Oospora.

Group of Leptothrix.

The members of this group occur chiefly as inhabitants of water. They are colorless, non-branching, filamentous microorganisms, morphologically closely related to Beggiatoa and Thiothrix. A small number of species only are known to occur in human beings and higher

animals either as superficial parasites of little or no pathological interest, or as occasional invaders of the internal organs where they may be associated with definite pathological lesions.

Leptothrix Innominata (Miller).—The organism occurs constantly in the buccal cavity, chiefly in the deposit about the teeth in human beings. It consists of unbranching curved threads averaging from 0.5 to 0.8 μ in width. They are non-cultivatable, and give with iodine a faint yellow color. Certain pathological conditions, including the production of caries in the teeth, have been attributed to this organism—a belief, however, which needs further confirmation.

Bacillus Buccalis Maximus (Miller).—Bacilli appearing singly, in threads, or in bundles, which are usually disposed in parallel lines from 30 to 150 μ in length. The individuals vary from 1 to 1.3 μ in thickness. The condition denominated pharyngomycosis leptothrica, which is characterized by the appearance of white, prominent, hard spots or nodules in the mucous membrane of the throat, and especially in the tonsils, has been attributed to this organism (Stern, Ackermann, and Chiari). The white masses consist of bundles of threads such as have been described, and give a characteristic iodine reaction. It has never been definitely proved that this organism is the cause of any pathological process.

Leptothrix Epidermidis.—Found by Bizzozero upon the skin of healthy human beings. The growth consists of non-branching, jointed threads, often convoluted and forming masses. In young cultures shorter rods may be found. The young rods are motile. Staining of the organism succeeds with all anilin stains and according to Gram. No blue color is developed with iodine. Growth takes place best in the presence of oxygen and is readily obtained upon all culture media. So far as is known, this species is of no pathogenic significance.

Leptothrix Cuniculi (Schmorl). *Synonyms.*—Streptothrix cuniculi (Kruse); Bacillus necrophorus (Flügge); Bacillus diphtheriæ vitulorum (Loeffler); Nekrosebacillus (Bang). Obtained by Schmorl from an infectious disease of rabbits. The disease begins about the lips and extends into the surrounding connective tissue, where necrosis is produced, associated with fibrinous inflammations of the serous membranes and pneumonia. A similar organism is said to occur in the diphtheria of calves, in the gangrenous pock of the cow, and in other pathological conditions in cattle, horses, and swine, and also to be sometimes present in the small intestines of swine (Kruse). As branching in this organism has not yet been proven to exist, it is for the present grouped under this heading. Cultivation succeeds upon blood serum under anaërobic conditions. Pure cultures of the

organism inoculated into rabbits produce a disease which cannot be distinguished from the spontaneous form. Other animals, guinea-pigs, dogs, cats, pigeons, and chickens, are insusceptible. Mice respond to inoculation. In guinea-pigs the organism multiples, if inoculated along with the pyogenic cocci. Similar epidemics in this country have been seen in rabbits, and a leptothrix agreeing with that described by Schmorl has been found in cover slips but never cultivated.

Leptothrix Filiformis (Flexner). *Synonym.*—Bacillus pyogenes filiformis. Obtained by Flexner in 1895 from the inflamed uterus as well as from the pleural and pericardial cavities of a rabbit which had died spontaneously. Afterwards found by Muscatello in Italy.

Morphology and Cultural Properties.—The organism as it appears in pathological exudates varies in length from 1.4 to 154 μ, the majority of the longer forms varying from 56 to 70 μ. The width varies from 0.5 to 0.7 μ. The thread-like bacilli stain irregularly, and seem to be composed of longer and shorter strands of protoplasm, which, as compared with the brightly staining chromatic points, take on a very faint color with anilin dyes. The chromatic areas are cylindrical rather than globular in form. The ends of the bacilli are rounded, and the longer forms show no evidence of transverse segmentation. Staining is readily effected with all the anilin dyes; but Gram's and Weigert's methods are negative. No branching takes place. The bacilli are non-motile. Their thermal death-point is 55° C., in five minutes. Drying also quickly kills the organisms. Spores are not formed. Cultivation upon ordinary cultural media, either in the air or under anaërobic conditions, does not succeed. Growth, however, can be obtained by employing the sterile organs of the rabbit; it has appeared by the end of from forty-eight to seventy-two hours, and is indicated by an opaque, delicate surface membrane covering the tissues. Growth takes place on these organs in an atmosphere of hydrogen, but somewhat less vigorously than in the presence of air. A small number of generations only can be obtained upon transplantation from organ to organ. The best medium of all as yet employed has been found to be the fœtus of the rabbit removed with aseptic precautions and placed in sterile tubes. Growth has taken place upon these in the thermostat in twenty-four hours, and a series of transplantations has been successful. The organisms, as obtained from artificial cultures, are somewhat shorter than those from the animal, and many more of the very short forms are present; they are also somewhat more slender, and have a tendency to stain solidly rather than in the regularly dotted way described.

Pathogenicity.—Suspensions of the original material containing

the bacteria obtained from the original rabbit were injected into the pleural cavity of others. An acute pleurisy and pericarditis developed, the animals surviving on an average about five days. At autopsy the serous surfaces were covered with a shaggy membrane which upon microscopic examination was found to consist of the bacterial growth. Intraperitoneal are less certain than intrapleural inoculations. Submeningeal injections produce meningitis and death. When introduced under the skin the material may give rise to no symptoms, whereas intravenous inoculations produce widespread abscesses. These were found constantly in the brain and heart muscle, occasionally in the liver, more rarely still in the voluntary muscles, and never in the kidney, spleen, or lungs. General infection with the organism rarely takes place. Guinea-pigs are relatively refractory. Although they succumb to the organism when introduced into the pleural cavity, they fail to respond in any way to subcutaneous injections. Mice and pigeons resist both intrapleural and subcutaneous inoculations. Dogs are insusceptible even to intravenous inoculations. The lesions consist of ordinary inflammatory exudates and extensive necrosis of the tissue at the site of development and even at a distance from the growth. The relation of this organism to leptothrix cuniculi is undetermined.

Leptothrix Asteroides (Flexner).—Described in 1900 by Cozzolino.[36] Obtained from a tumor in the neighborhood of the ear, and from a metastatic retropharyngeal abscess in a young woman. The organism as obtained from these situations appears in the form of minute grains of yellow or reddish color.

Morphology and Cultural Properties.—The microorganism composing the grains consists of bundles of filamentous bacteria. Some are folded or bent so that they take the form of spirilla. They stain with the ordinary dyes and by Gram's method. The cultures show marked polymorphism. In agar or serum cultures the bacillary elements vary from 2 to 5 μ in length, many of the threads being made up of several bacilli joined together. These pseudo-threads are apt to occur more especially in moist media. The spores appear in the interior of the rods; sporulation being increased as the drying of the medium goes on. The spore-containing bacilli resist a temperature of 100° C. for fifteen minutes. In special media, as for example in bouillon made of grits and in gelatin, growth appears in strands of interlacing fibrils. Branching does not occur. The optimal temperature is between 37° and 40° C., but growth can still take place between 40° and 60° C. The organism is facultative anaërobic. At the room temperature development is very slow and may fail altogether. A faintly alkaline reaction of the medium is desirable. Growth upon

all favorable media consists of a continuous membrane which appears dry, white, and resistant. Bouillon is clouded in from twelve to eighteen hours, but after the formation of the membrane it becomes clear by the sedimentation of the growth. On the surface of agar stabs a crumpled membrane is formed, and from the sides of the thread-like growth hair-like processes extend into the medium. This latter feature is augmented upon agar plates. Gelatin is slowly liquefied. On potato the growth is active at the temperature of the thermostat, and a crumpled membrane is formed. Blood serum is slowly peptonized. Upon agar pigmentation varying from a rose tint to a deep brown appears, while upon serum a wine-red color is produced. The pigment is soluble in water, less so in alcohol, and is insoluble in ether and chloroform. Lactose is not fermented, nor is an acid formed. Indol is not produced.

Pathogenicity.—Young guinea-pigs and house mice are susceptible, whereas white mice and rabbits are refractory. The most certain results are obtained by injections into guinea-pigs, made into the peritoneal or pleural cavities. The organism in fatal cases can be cultivated from the seat of the inoculation and also from the blood. The most striking lesions in the body consist of necroses in the liver and congestion of the lungs. The swelling near the ear, described as a tumor, from which the organism was originally obtained, resembled the lesions produced by streptothrix actinomyces. In sections of the original swollen tissue it was possible to demonstrate a network of threads, with peripheral edges extending as rays into the tissue, upon which structures resembling the clubs developing upon the peripheries of the actinomyces organism occurred.

The Group of Cladothrix.

This group is at present almost entirely without medical interest, the pathogenic organisms formerly assigned to it having been found to belong to the streptothricees. One or two doubtful examples may still be included here. Naunyn described one such organism, which he found in a hemorrhagic infiltrated area in the cerebral meninges and upon the endocardial excrescences in a case of chorea. Zopf regarded it as occupying an intermediate position between the cladothrix and leptothrix forms of microorganisms. Baumgarten and Neumann have drawn attention to the fact that at the time of the isolation of this organism the water supply which had been used for washing off the organs contained large numbers of microorganisms similar to those observed by Naunyn upon the cerebral membranes and upon the heart valves. Rabe described as a clado-

thrix an organism which he obtained from purulent phlegmons in a dog. He observed in two dogs a diffuse inflammation of the skin and subcutaneous tissues of one of the forepaws, which in the first case underwent partial necrosis, and in the other dog gave rise to suppuration of the superficial cervical glands. A third animal, which about three months previously had had an abscess of the parotid gland, died after four days of an acute peritonitis. In the last animal, as well as in the two previous ones, Rabe found in the pus many grape-like, lobulated, pale granules which upon magnification resembled the actinomyces organism. Cultivation experiments failed, but the inoculation of goats and rabbits gave, according to Rabe, positive results. It was possible by the use of infected material to produce local abscesses which, however, usually healed, although in one instance they caused the death of the experimental animal in six days. In the first case only was the organism recovered from the site of the inoculation. The imperfect description of this organism does not permit of a definite conclusion as to whether it was a genuine cladothrix or whether it belonged to the streptothrix group.

Cladothrices are inhabitants of water. They are colorless, filiform bacteria, free from sulphur, that can be shown upon careful and proper staining to consist of superimposed rods. Displacement of single bacilli of the chains and growth in a new direction gives rise to appearances suggesting branching (pseudo-ramification). The repetition of this process brings about multiple branchings, and the intertwining of the branches produces convoluted filiform masses. Multiplication has been described through the medium of conidia, that are homologous with rods of other bacterial species. Zopf has observed in cladothrix dichotoma the transformation of certain members of the threads into four or five round bodies resembling cocci. The best example of this group is C. dichotoma, discovered by F. Cohn, which is a constant inhabitant of fresh and stagnant water. It is of no medical importance.

The Group of Streptothrix.

In cultures growth takes place as sterile threads with the production of mycelium; or the threads may sometimes divide into globular or chain-like round germinal cells (conidia, spores). These bodies have almost nothing in common with the resistant endogenous spores of the bacteria. While they are somewhat thicker and more retractive than the mycelia in which they develop, they stain in a similar manner, and it still remains to be proven that they are more durable and resistant than the vegetative threads themselves. They are, how-

ever, capable of reproducing the species. The mycelia consist of cylindrical, dichotomously branching threads, which lack a doubly contoured membrane. The hyphæ do not contain fluid and granular contents, nor are they separated from each other by partition walls. In structure the threads agree with the filamentous bacteria.

STREPTOTHRIX ACTINOMYCES. *Synonyms.* — Actinomyces bovis (Harz); A. bovis sulphureus (Gasperini); Nocardia actinomyces (De Toni and Trevisan); Oospora bovis (Sauvageau and Radais); Actinomyces fungus. This organism is frequently alluded to as the ray fungus. It was observed originally by v. Langenbeck in an unpublished case of fatal prevertebral abscess, and was figured by Leber in his celebrated atlas. It was obtained from the maxillary tumors of cattle by Rivolta and Perroncito, and from this source was first accurately described by Bollinger in 1877. The disease in human beings, corresponding to that in cattle, was first recognized by J. Israel in 1885. The first microscopical study, as well as the first description of cultures, we owe to Boström. J. Israel and Wolf later obtained cultures which, however, differed from those of Boström, since they grew only under anaërobic conditions. It is highly probable that several varieties or even species of the organism may be concerned with different examples of actinomycosis. Here the subject will be considered under one head, and no attempt will be made to separate the organism into species or varieties, since at the present time such a procedure seems unprofitable.

Morphology and Cultural Properties.—As the organism appears in human beings and in animals, it consists of rosettes varying from 0.2 to 0.6 or even up to 1.2 mm. in size. The color varies; some of the grains are gray, while others are yellow, red, or even green. These rosettes (called by the Germans *Drusen*) consist of interwoven threads which at the periphery show a radial arrangement. The more external portions of the threads, and more rarely those in the interior, show flask-shaped thickenings which have a high degree of refraction and staining-properties different from those of the rest of the thread. Sometimes instead of these bulbous swellings simple nodular endings of a similar nature may be found. The threads vary from 0.4 to 0.6 μ in diameter. They are usually non-membranous, and may be seen to give off side branches, and secondary branches in which there is protoplasmic continuity throughout. Sometimes the threads are made up of a succession of shorter rod-like structures. In the interior of the rosettes spherical coccus-like bodies are sometimes found, which may be shown to arise through successive rapid division of the filaments. Sometimes the swellings at the ends of the filaments may be entirely wanting; this is a quite common observation in the case

of the pale gray translucent grains occurring in pus. Staining is possible by means of the ordinary anilin dyes, and excellent results are obtained with Gram's and Weigert's methods. In order to demonstrate the bulbous extremities double staining may be used. Several methods are applicable. In dealing with tissues the sections may be stained first in carmine, or deeply in eosin, and then afterwards by Weigert and Gram in gentian violet. A better method—one which has recently been recommended by Mallory—consists in staining the section in gentian violet on the slide, after which the bleaching and dehydrating are accomplished with anilin oil, in which a small amount of basic fuchsin has been dissolved. When the decolorization has been carried sufficiently far, the excess of the anilin-oil fuchsin is removed with xylol and the section is mounted in balsam. By these several procedures the filaments and coccus-like structures appear blue, whereas the hyaline bulbous extremities are sharply differentiated in red. Similar methods of procedure may also be employed in the double staining of cultures. Boström has shown that these swollen extremities are to be regarded as representing degeneration products, and consist of a kind of colloid transformation of an outer membrane about the threads. Cultures are obtained with some difficulty inasmuch as many of the grains observed are dead. It is best before making inoculations of the culture media to triturate the grains in a sterile mortar. Growth takes place, according to Boström, upon agar, serum, gelatin, and bouillon, best in the thermostat, but also at the room temperature. In pus obtained from infected animals other microorganisms are sometimes present, and more difficulty is experienced in obtaining the cultures. The young colonies appear as grayish points consisting of rays and of a network of fibrils. The older colonies are more opaque, and the peripheries distinctly fibrillar. Growth takes place best under aërobic but also under anaërobic conditions. Upon blood serum the colonies sometimes assume a yellowish-orange or brick-red color. At first they are separate, but may later become united through continuous growth. Gelatin is slowly liquefied. Growth takes place also upon potato along the line of inoculation, and is of a yellow or reddish color. Bouillon remains clear, the colonies accumulating at the bottom of the tube. These colonies are composed of balls of interwoven threads in which bulbous structures cannot be demonstrated (Lehmann and Neumann). Upon the surface of solid cultures a branching mycelium is formed, whereas in the depth the bulbous swellings appear. The spherical coccus-like structures spoken of as "spores" are found on the surface of the growth, constituting a white layer which can be removed by shaking. These upon transplantation give rise to

another generation. They differ from ordinary spores in showing staining properties similar to those of the protoplasm of the bacterial cell. Cultures may remain alive for a long time (nine months, Lehmann and Neumann). Heating to 75° C. for five minutes kills them (Domec).

It should be mentioned here that the organism cultivated from two cases of human actinomycosis by J. Israel and Wolf differed in some respects from the foregoing. In the first place development was better under anaërobic conditions, and secondly, there was an absence of branching threads in ordinary culture media. According to Gasperini, continued cultivation of the organism outside of the body is associated with a more pronounced aërobic character, and moreover, as it loses its capacity to grow anaërobically, the virulence also becomes weakened.

Pathogenicity.—The organism has not thus far been found in external nature, but there is much reason to believe that it occurs upon various grains and perhaps grasses, since the greater number of infections have been associated with the entrance of these substances into the body. They have been found in the actinomycotic tumors by Boström. Piana observed repeatedly fibrous bundles of grain in the actinomycotic nodules of the tongue in cattle. In human beings infection has also been found associated with the presence or entrance of grains into the body. Soltmann reports a case following the accidental swallowing of a piece of barley straw, and more recently Ammentorp observed an instance of actinomycosis of the abdominal cavity, in which the primary focus of infection had been in the appendix vermiformis. In the contents of the abscess a barley grain was found. The common occurrence of the infection about the mouth and head and the respiratory tract, both in animals and man, speaks also for this mode of infection. There is no evidence of the transmission of the disease from cattle to man or from one human being to another. The infection would appear to be direct in all instances. According to the point of entrance and development of the parasite the primary infections are distinguished into oral, pharyngeal, respiratory, intestinal, cutaneous, etc. The mouth and pharynx are most commonly infected; and next in order, the abdominal cavity and lungs. More unusual primary foci of localization are the larynx (Mündler), and the appendix vermiformis (Zaufal, Ammentorp, Latimer, and others). Primary infection of the respiratory tract occurs in two forms. Thus Canali has described a superficial inflammation of the bronchi which in its clinical course resembles a chronic diffuse bronchial catarrh. In the secretion the actinomyces rosettes were found. More important are the parenchymatous involvements

in which, besides inflammation of the bronchi, typical invasion of the parenchyma of the lung is present. The inflammation of the lung is usually of the lobular type, but not uncommonly abscesses are developed which may be evacuated through the bronchi and leave behind definite cavities. The sputum as well as the inflamed lung tissue contains the rosettes. The writer has observed two such instances in which adhesions between the lung and the chest wall had taken place with the formation of sinuses communicating with the surface of the body. From the contents of these fistulæ the rosettes were obtained during life as well as from the bronchial secretion. In addition to the characteristic rosettes fragments of the parasite are also found in the lung amid the diffuse products of inflammation. The acute inflammations and suppurations of the lung are associated with chronic interstitial inflammations in adjacent portions of the lung tissue.

Besides the form of intestinal implication observed in cases of appendicitis, other portions of the intestinal tract may become infected. Baumgarten distinguishes two forms, one superficial, the other a so-called destructive parenchymatous affection. The latter, which is the more important, is characterized by the appearance of isolated foci, the size of a pea or a bean, occurring in the submucous tissue and sometimes in the mucous membrane, which may undergo ulceration with the production of fistulous tracts. These ulcers may be sufficiently deep to reach the muscularis, and inflammations may take place in the peritoneal coat uniting the adjacent loops of the intestine, or adhesions to the parietes of the abdominal cavity may be formed, the ulceration even extending to the outside. From the foci in the lung as well as from those in the intestinal tract or from other foci of infection metastases may occur, and other organs, the liver, the joints, the kidneys, the heart, or the brain, may become the seat of secondary developments; or by continuity, in primary respiratory or intestinal forms of infection, the peritoneum, the pleuræ, or the pericardium may become the seat of inflammation. Bollinger has described a cryptogenic infection. In his case there appeared to be a primary infection of the brain. No point of entrance of the organism could be made out.

In animals the pathological process is characterized by a new formation of connective tissue, whereas in human beings suppuration is more likely to occur. The actinomycotic processes, therefore, in the lower animals partake more of the characters of tumor, and have to be differentiated more especially from the sarcomata. This point can readily be settled by the discovery of the parasite. Of cattle the ox is the most susceptible, the disease occurring more rarely in swine,

dogs, and horses. The primary focus is most commonly in the medulla of the maxillary bone; at first a cellular granulation tissue, and later a thick fibrous tissue develops in this situation. The medullary canal enlarges and a new growth of bone takes place from the periosteum. In rare cases the soft tissues about these bony parts are first affected, and the latter are invaded only secondarily.

Inoculation into the lower animals has given very inconstant results. Boström did not find any increase of the parasites, encapsulation and eventually absorption taking place. If large numbers of rosettes are introduced, multiple nodules may develop in the serous cavities. A microscopical examination of these structures shows that the filaments have entirely disappeared, the clubbed extremities alone remaining. Israel and Wolf claim to have obtained positive results with their cultures inoculated into rabbits and guinea-pigs. After intraperitoneal inoculations there developed within a period varying from four to seven weeks tumors ranging in size from a millet-seed to a plum, which upon section were found to consist of a fibrous mass enclosing tallow-like contents. In these typical rosettes with bulbous ends were present. Cultures were obtained from these structures, and other animals were inoculated successfully.

Streptothrix Farcinica. *Synonyms.* — Bacille du farcin des bœufs (Nocard); Nocardia farcinica (de Toni and Trevisan); Actinomyces bovis farcinicus (Gasperini); Oospora farcinica (Sauvageau and Radais). Discovered by Nocard in a disease of cattle that rarely appears in France. The lesions consist of nodules that are suggestive of farcy, or of tuberculous affections of the superficial lymph glands. Similar nodules may occur in the internal organs. The organisms appear as branching threads, made up of relatively short members. Motility is absent. Staining is positive with ordinary anilin dyes and also by Weigert's method. Growth is obtained upon ordinary culture media, including potato and milk. Colonies upon agar appear as yellowish-white, irregularly outlined, refractive, membranous points, averaging from 1 to 2 mm. in diameter. Temperatures from 30° to 40° C. and the presence of air are required. The deep colonies are lens-shaped. According to Nocard unstainable spores appear, a statement which Lehmann and Neumann could not confirm. Guinea-pigs, cattle, and sheep are susceptible to inoculation, while rabbits, dogs, cats, horses, and donkeys appear to be immune. Intraperitoneal inoculations of pure cultures of pus containing the organism produce in guinea-pigs, at the end of from nine to twenty days, extensive pseudotuberculosis of the omentum. Intravenous inoculation gives rise to nodules in the various organs. After subcutaneous inoculation infection is limited to the adjacent lymph glands. In the centres of

the nodules a filamentous streptothrix is demonstrable. Clubbed extremities appear not to develop in this organism.

Streptothrix Maduræ.—This organism, which has yet to be distinguished from S. actinomyces, is found in mycetoma or madura foot. The disease is characterized by suppurating nodular swellings, enlargement and distortion of the foot, the bones, when involved, undergoing a rarefying osteitis. Besides the feet the hands may also be affected. The disease is common in the East Indies, and instances have been observed in North America and Italy. Bristowe and Carter in England described in the diseased tissues yellow or black granules consisting of a network of mycelial threads. Kanthack, Boyce, Surveyor, and Hewlett pointed out the similarity with the organism of actinomyces and identified the two processes. Two different kinds of granules are associated with two varieties or forms of the disease, namely, the melanoid or black variety, and the ochroid or pale variety.

Carter believed that he had cultivated the fungus which grew as a pink mould. Vincent cultivated the granules from the pale form of the disease, and found the growth to be made up of a streptothrix which grows with a marked red color upon potato. He describes it as consisting of branching threads, varying from 1 to 1.5 μ in thickness, upon the surface of which as well as in the depth spores developed, varying from 1.5 to 2 μ in size. Staining is accomplished with the ordinary anilin dyes and by Gram. The threads are killed in from three to five minutes at 60° C., the spores succumbing only at 85° C. Growth takes place at the ordinary temperatures, best at 37° C., and only in the presence of oxygen. Glycerin-agar is a good medium. Gelatin, which may also be used, is not liquefied. The colonies consist of granules, at first yellow, white, and later of a red color, which are of striking hardness. Infusions of hay, straw, potatoes, and other vegetables give flourishing growths. A membrane is formed covered by a sporogenous layer. Animal experiments have failed. The parasite is found often associated with pus organisms in a cellular, sometimes hemorrhagic, very vascular granulation tissue. Bulbous ends are stated not to appear. J. H. Wright expresses the opinion that the cases of the ochroid variety of mycetoma are probably examples of actinomycosis. The black granules have been studied, and cultivations have been successfully made by this investigator. The granules are of an irregular or mulberry or black or dark brown color, and usually less than 1 mm. in diameter. They are hard and difficult to break up under the cover glass. Microscopically they present the appearance of an irregular lobate mass of dark brown or black, opaque substances. Under the high power of the microscope typical

septate branching hyphæ, sometimes showing dilatations or varicosities of the segments, may be seen. The periphery of the granules appears to be made up of closely set, radiating hyphæ, showing more or less swelling or degeneration of the segments. From these a growth of hyphomycete has been obtained. In all cases the growth in bouillon began from the grain and appeared after four to five days or even later. On solid culture media the growth first appears as a tuft of delicate whitish filaments springing from the black grains. After some days an increase takes place until, in the case of the potato, there is produced a dense whitish or pale brown feltwork or membrane having a tendency to spread widely. The organism consists of long branching hyphæ from 3 to 8 μ in diameter. The young ones show delicate transverse septa, while in the older ones swellings have developed at these points, so that the hyphæ appear as a string of oval-ended plump segments. The filaments have a definite wall, and in their interior granules or pale areas may be seen. Branching occurs by the formation of lateral outgrowths or buds. No spore-bearing organisms have been observed. In old cultures, grown in potato infusion, numerous black granules about 1 mm. in diameter appear in the midst of the mycelium. These masses consist of a sclerotium. Animal experiments have entirely without effect. From this study it would appear as if mycetoma was produced by two distinct parasites and really consisted of two diseases; the pale variety probably being nothing else than actinomycosis and the black a hyphomycetic infection.

Streptothrix Eppingeri. *Synonyms.* — Cladothrix asteroides (Eppinger); Oospora asteroides (Sauvageau and Radais). Obtained by Eppinger in pure culture from an abscess of the brain which had led to cerebrospinal meningitis. The organisms were found also in the bronchial and supraclavicular lymphatic glands in the same case. They appear as branching threads averaging 0.2 μ in thickness. They are stainable by Gram's method. Some of the threads show a division into short, quadratic, coccus-like members. They grow upon ordinary culture media; upon potatoes, according to Eppinger, spores are produced. Lehmann and Neumann were not able to obtain spores in their cultures. The optimum growth is at 37° C., and the best medium is two per cent. glucose-agar. The individual colonies develop best upon the surface of the medium; they are yellowish-white in color and dull in appearance, presenting a finely granular nucleus, and a thin, pale, concentric outer zone. Gelatin is not liquefied. The short threads have a slow motility, whereas the very short threads and coccus forms are quite active (Eppinger). According to Lehmann and Neumann, however, motility is absent. Rabbits

are susceptible to intravenous and even to subcutaneous inoculation. Guinea-pigs succumb to both subcutaneous and intraperitoneal injections, and in both at the end of from five days to four weeks a pseudo-tuberculosis of the various organs can be noted. Mice are refractory. The pseudo-tubercles consist of "leucocytes without giant cells," and show caseous centres in which the streptothrix threads can be demonstrated. Artificial cultivation causes the organism quickly to lose its virulence.

Streptothrix Pseudotuberculosa (Flexner).—Obtained in 1897 from a diffusely consolidated and caseous lung suggestive of a tuberculous caseous pneumonia, in a man. The pathological process had consisted mainly in an inflammatory exudation which had undergone caseation, but in addition circumscribed nodules having the size, form, and appearance of miliary tubercles were present in small numbers. These nodules were composed chiefly of epithelioid cells, some of which were disintegrating. Giant cells were not seen. Similar nodules were present in the abdominal cavity, especially in the omentum which was much thickened. They contained giant cells as well as epithelioid cells. The microorganism, which consisted of a streptothrix appearing in the form of more or less convoluted masses, only rarely as single branching filaments, was very abundant in the lungs. The organism in the peritoneal nodules was of a somewhat simpler structure. The contours of the rods are slightly irregular, the staining is not quite uniform, and very rarely just before its termination a filament will show two or three cross partitions, suggesting the breaking up into as many short rods. Coccus-like forms were not encountered. Staining is accomplished best by Weigert's method, after which the rods show some irregularity in that deeply stained chromatic granules or particles may be separated from bits of unstained protoplasm of greater diameter than the stained particles. Cultures were not obtained, perhaps for the reason that a vigorous growth of a bacillus identified as belonging to the group of B. coli communis had developed on all cultures from the lung. Guinea-pigs inoculated subcutaneously with some of the material from the lung did not react. A similar organism has been described by Buchholz, who obtained it in Berlin from the lung of a man which was consolidated and contained cavities. The morphology of the organism is identical with that of the organism described by Flexner. Buchholz also failed to obtain cultures. Scheele and Petruschky described a streptothrix obtained from a woman which is probably similar to those described. It was present in the lungs and in several subcutaneous tumors which had undergone suppuration. It consisted of fine, branching threads, but there were no evidences of fructification.

Larkin and Norris in Prudden's laboratory observed two cases of pulmonary infection due to a similar streptothrix.

Besides the above unclassified streptothrices several additional ones have been obtained from morbid conditions in human beings. Rosenbach found a branching microorganism capable of growing upon artificial culture media, and of reproducing upon inoculation a similar pathological condition in a case of so-called erysipeloid (erythema exsudativum multiforme). Garten obtained in impure culture a branching microorganism from abscesses, and a fistulous tract extending from the upper dorsal vertebra to the sacrum. He regards the organism as a new species, but has failed to establish its distinction from S. actinomyces. Additional instances of streptothrix infection are reported by Sabrazès and Rivière, and by Ferré and Faguet. The first case, of Sabrazès and Rivière, occurred in a man in whom abscesses were found in the brain, lungs, and kidney (softened infarction). The pus from the brain abscess showed single and radiating branching threads; in the kidney the filaments were much shorter, while in the lungs neither form was discovered. In cultures cocci were found associated with the streptothrix, the latter growing anaërobically only. In a second case these observers isolated from sputa and from a subcutaneous abscess in a man an aërobic streptothrix, pathogenic for small animals. Ferré and Faguet obtained in a pure culture from an abscess of the brain in an epileptic a streptothrix, which proved, however, to be non-pathogenic for guinea-pigs and rabbits. In how far these represent new species is yet to be determined.

Bibliographical References.

Owing to the fact that a considerable part of this article was written while separated from my library, during a temporary residence abroad, very few references have been introduced into the text. Those here given cover some of the current books on the subject of bacteriology which have been consulted, and a few special articles that were used in the completion of the article only. General acknowledgment is made to the current literature on bacteriology and pathology.

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MICROORGANISMS (PROTOZOA).

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MICROORGANISMS (PROTOZOA).

THE protozoa form the lowest division of the animal kingdom and include those organisms which consist of a single cell. In the biological scale they separate the more complicated multicellular animals from the unicellular members of the vegetable kingdom. But here, as elsewhere in organic nature, the lines of division are not sharply drawn, and on the one hand, unicellular organisms may cohere to form aggregations or colonies resembling the multicellular forms, while on the other hand, the distinguishing between unicellular animals and unicellular plants has been found so difficult that some naturalists would relegate all unicellular organisms to a third indifferent kingdom.

In view of these difficulties, Butschli, in his classical work upon protozoa, defines them as organisms consisting of a simple cell or of a colony of similarly constructed simple cells, and resembling in their physiological manifestations—food ingestion and metabolism, irritability and movement—the typical multicellular animals. The parasites with which we have to deal present so definitely the physiological and morphological peculiarities of animals that no difficulty arises in determining to which kingdom they should be assigned. Certain general anatomical and physiological characteristics of unicellular animals may be considered.

In the great majority of instances the cell, consisting of living protoplasm varying considerably in its physical characters, contains a well-defined body, the nucleus, composed of material which differs in character from the remainder of the cell substance. In certain protozoa no nucleus has been demonstrated, though in many forms in which it was formerly supposed that no such structure existed, more careful examination with the aid of improved methods has revealed its presence. In many instances, however, the nucleus has not the typical form observed in the cells of higher animals, namely, a clear vesicular body containing particles of the characteristic nuclear substance, chromatin. In certain protozoa, for example, the nucleus is represented by a homogeneous body composed of material which stains more readily than the remainder of the cell. The fact that in some infusoria the nuclear material may be disseminated throughout

the cell body in the form of small particles has thrown doubt upon the absence of a nucleus in other forms in which as yet none has been demonstrated.

The cell body presents the greatest varieties of form. Many functions being performed by the single cell, its structure presents numerous modifications in conformity with its various activities. Commonly, however, a peripheral zone of protoplasm clear and hyaline in appearance can be distinguished. This is spoken of as *ectosarc*, while the central portion containing the nucleus is granular and is termed *endosarc*.

Many protozoa are surrounded by a cuticle or shell which serves as a protective covering. Its formation may be due to a modification of the external layer of protoplasm which has become denser and more resistant to external influences; in other cases the cuticle may be composed of some substance differing chemically from the cell protoplasm and separable from it. Thus it may consist of a soft chitinous substance or be formed mainly of calcium carbonate or silica, and presents in different species the greatest diversity of form. Certain protozoa when exposed to adverse external conditions are capable of forming about themselves a temporary protective covering and are then said to be encysted. Many forms, notably the coccidia in the stage immediately preceding that of reproductive division, become encysted, and segmentation of the protoplasm takes place within the capsule.

The majority of protozoa are capable of independent motion. This, in many instances, is due to the ability of the organism to cause a local modification of its shape by the protrusion of a protoplasmic process at any point upon its surface. As more and more of the cell body flows into a protrusion of this kind the whole organism gradually changes its position and from continuous thrusting out of a process in one direction a progressive movement of the body results. Other protozoa are provided with specially formed organs of locomotion—flagella or cilia. From the periphery of the cell may project one or several long thread-like filaments or flagella capable of active lashing movements which serve as a means of propulsion, or an organism may be provided with numerous similar but very short projections called cilia, having a rhythmically vibrating movement.

Like higher animals, the protozoa are in most cases capable of ingesting solid particles of food which may be taken into the cell body at any point upon the periphery by means of pseudopodia thrust out about them or may be received at some point specially fitted for their reception, a mouth, provided, perhaps, with cilia which direct a current of fluid towards an opening. Once in the body, the food

particle is often found lying within a clear area or vacuole until it is dissolved and assimilated. In many cases, however, food ingestion is like that of plants, nutrient material reaching the organism by osmosis from the surrounding media. The so-called contractile vacuole, capable of alternate contraction and expansion, is present in certain protozoa and is believed to be concerned with the elimination of waste products from the organism.

Like tissue cells, the protozoa increase in size by food assimilation, and having attained a certain volume divide into two or more parts, each of which repeats the development of the parent. Most protozoa are capable of simple fission, direct or amitotic division of the nucleus preceding division of the cell body, with formation of two new individuals. A second method of reproduction is very frequently observed; the organism, usually after becoming encysted, divides into a greater or smaller number, from four or five to a hundred or more small bodies. These bodies usually acquire a protective covering and are spoken of as *spores*. The protoplasmic contents may divide into two or more reproductive bodies, usually sickle-shaped, each of which, after its liberation by rupture of the capsule, is capable of reproducing the life history of the organism. In many instances as a preliminary process to this multiplication two adult organisms can be observed to approach one another, and as a result of their contact the substance of one merges with that of the other, the two nuclei forming a single nucleus. This process of fusion is called conjugation. At times peculiarities of size or structure distinguish the two conjugating organisms which in a rudimentary way represent sexually distinct individuals. Among certain ciliata a modification of the process occurs. Two adult organisms come in contact, and an interchange of nuclear material takes place; the organisms separate and subsequently undergo reproductive multiplication.

Classification.

The classification of the protozoa most widely accepted is that adopted by Butschli; he distinguishes four classes: (I.) Sarcodina —organisms characterized by an amœboid form and the ability to protrude protoplasmic processes. (II.) Sporozoa—parasitic organisms which reproduce by division into a varying, often large, number of segments. (III.) Matigiphoria—protozoa which possess one or several flagella. (IV.) Infusoria—protozoa, characterized by possession of numerous cilia.

As compared with bacteria, the number of protozoa parasitic in man is very small, and the number of diseases which have been

proven to be caused by protozoan microorganisms is even more limited. They are parasitic in considerable variety in the lower animals, and in the present connection certain forms are important in that they throw light upon the life history of closely related human parasites or cause important diseases in domestic animals. The methods at our disposal for the study of these microorganisms are much more limited than those employed in the separation of bacterial species. Cultivation outside of the animal body in which they live can be effected, if at all, only with the greatest difficulty. In studying the etiological relation of these parasites to disease reliance must be placed upon the demonstration of a constant association of the organisms with the disease in question, of its relation to the lesions of the disease, and upon the result of the inoculation into other animals of material containing the organism. In fresh and stained preparations from the fluids and tissues of the affected animal it is possible to study the morphology and life history of the parasite within the body.

I. Class: Sarcodina.

The Sarcodina are protozoa which during the chief period of their active or motile existence change their shape by protrusion of non-vibratile protoplasmic processes and are thus capable of changing their position or ingesting food materials. The processes may show the greatest variety of form, from the short and blunt protrusions seen in the amœba to long, delicate filaments anastomosing with one another and capable of but little change of shape. Various intermediate forms are seen, and upon the peculiarities presented by these the organs as a basis, the class is divided into a number of orders: (1) Rhizopodia; (2) Heliozoa; (3) Radiolaria.

Amœbæ.

In the study of parasitic microorganisms we are concerned only with the Rhizopodia or Amœbæ—protoplasmic bodies, naked or with a shell, capable of protruding short, blunt processes. The presence or absence of a shell distinguishes the sub-orders, Testacea and Nuda, the latter including the parasitic forms.

Amœbæ are at times found in the intestines of healthy human beings. For us the most important of the group is a microorganism indistinguishable morphologically from the amœba of the normal intestine but constantly associated with one variety of dysentery which has been designated amœbic dysentery. Amœbæ of a similar

character have been observed by Bälz, Jürgens, Kartulis, and Posner in the human bladder. Flexner has found amœbæ in the pus from a buccal abscess involving the submaxillary bone; and Kartulis has made a similar observation in a case of osteomyelitis of the submaxillary bone.

Amœba Coli (Lösch). *Synonym.*—Amœba dysenteriæ (Councilman and Lafleur).

Amœbæ were found in 1859 by Lambl in the intestinal evacuations of a child suffering with enteritis, but as they were associated with other organisms he did not regard their presence as important. Subsequently, Cunningham found amœbæ in the stools of a considerable number of cholera patients. Amœbæ were observed by Lösch in 1875 in the dejections of a dysenteric patient in whom extensive ulceration of the colon was found at autopsy. Lösch described carefully the organism, giving it the name *Amœba coli*, and for the first time ascribed to it an etiological relationship with the dysentery. In sections through the base of intestinal ulcers from cases of dysentery observed in Egypt, Koch found amœbæ and came to the conclusion that they were the cause of the disease. Kartulis was able to demonstrate their presence in the stools of over five hundred cases of Egyptian dysentery, while noting their absence in other intestinal disorders, as in typhoid fever and tuberculosis. He also found them in the pus and in the walls of the liver abscesses which are frequently associated with this form of dysentery. Subsequent observations have proven the organism to have a wide geographical distribution. Osler was the first in America to observe amœbæ in the stools of a patient suffering with dysentery and in the pus of an associated liver abscess. In Italy, Germany, Greece, India, and South America similar observations have been made. The pathology of that form of intestinal flux which is associated with the presence of amœbæ in the stools and in the wall of the intestinal lesion has been carefully studied by Councilman and Lafleur, Kruse and Pasquale, and others.

Amœbæ have, however, been found by a number of observers in the stools of normal individuals. Cunningham observed them in normal stools and in those of cholera patients, and came to the conclusion that fluidity and alkalinity of the evacuations favor their presence. Of twenty individuals to whom Schuberg administered salines, amœbæ were found in the dejections of one-half, but were absent in solid fæcal material. They have been found in healthy individuals and in those suffering from a variety of intestinal diseases by Grassi, who thinks that they are in Italy frequently parasitic in the intestine, though he ascribes to them no pathogenic importance. In Egypt, Kruse and Pasquale found the organism in a few cases in non-dysen-

teric individuals. They believe that parasitic amœbæ not associated with dysentery occur with varying frequency in different localities. The organisms found in healthy subjects and in those suffering from diseases other than dysentery present no morphological characteristics which serve to distinguish them from the Amœba coli of Lösch.

Morphology.—The amœbæ of dysentery vary in diameter from 10 to 35 μ. In fresh stools they may be recognized as very refractive bodies, very readily distinguishable, owing to their active amœboid movements, from the cells which may be present. A clear hyaline ectosarc forms an external zone about the very refractive granular endosarc. The distinction between endosarc and ectosarc, though conspicuous in actively moving organisms, is not always, in bodies which have assumed a spherical form and are at rest, to be made out. The activity of the amœboid movement varies considerably and is most conspicuous when fresh fæcal material is examined upon a warm stage. The movement consists in the thrusting out of blunt protoplasmic processes at first involving only the hyaline ectosarc, but with increasing size consisting subsequently of endosarc as well. The protrusion of such processes may cause merely a change of form and several thrust out at the same time may give the body considerable irregularity of outline. A progressive motion results from repeated protrusion of the ectosarc in some definite direction.

The endosarc contains clear round areas or vacuoles which vary much in size, occasionally having a diameter one-third that of the organism. Various foreign bodies may be enclosed within the cell, lying always within the internal zone. Red corpuscles are frequently seen; at times as many as six in a single cell. They may be well preserved and readily recognizable by their color, or they may appear as round decolorized bodies. Granules of pigment apparently derived from engulfed corpuscles are also found. Bacteria are occasionally observed within the cell body. Such foreign material, presumably ingested by means of pseudopodia, serves apparently as food for the organism, but it is not improbable that nutrient material is also obtained by absorption from the surrounding fluid medium.

The amœba possesses a nucleus which is only rarely demonstrable in fresh preparations, being with difficulty distinguishable from the vacuoles present, though the latter are somewhat less refractive and have a less distinct though more regular outline. In specimens which are fixed and stained the structure of the organism is more readily observed. The parasite may be studied in tissues which contain it or in preparations made from the fresh stool by spreading out a drop of mucus upon a cover glass and immersing in some fixing fluid. Mallory recommends thionin as a differential stain for amœbæ. The

internal portion corresponding to the endosarc stains more deeply than the outer zone; the nucleus is situated excentrically and appears as a round vesicular body, staining sharply at the periphery, and frequently containing in the centre a deeply stained nucleolus.

The exact method of reproduction has not been demonstrated. It is probable, however, that simple binary fission takes place, and although direct evidence of nuclear and cell division is wanting, at times pictures are seen which indicate the existence of such a process. Sporulation has not been observed.

Cultivation.—Attempts to cultivate the Amœba coli in artificial media have been attended by little, if any, success. Kartulis believed that he was able to obtain cultures of the organism by inoculating alkaline straw infusions with dysenteric fæcal material or with pus from the associated hepatic abscesses. At the end of twenty-four to forty-eight hours a thin membrane forms upon the surface of the infusion, kept at a temperature of 36° to 38° C., and preferably allowed to stand, according to Kartulis, in open vessels. The membrane is composed of bacteria and amœbæ much smaller than those found in the stools and without amœboid movements. On the following day they have grown to the size of ordinary amœbæ coli and are capable of progressive movement by the protrusion of pseudopodia. Small round non-motile nucleated bodies with double contour and about the size of white corpuscles were regarded by him as spores. Kruse and Pasquale repeated these experiments, but were unable to cultivate the Amœba coli in sterile straw infusions and found, indeed, that the implanted organisms rapidly disappeared. They observed, however, that in unsterilized straw infusions an amœba very closely resembling the organism cultivated by Kartulis develops in several days. The same small double contoured bodies, presumably spores, were found, and it is probable, as Kruse and Pasquale believe, that the organism grown by Kartulis is not the Amœba coli, but an organism which develops in incompletely sterilized straw infusions, or enters the uncovered vessels from the air, or is introduced in the form of spores with the inoculated material. Attempts have been made by Celli and Fiocci to cultivate the Amœba coli upon a solid medium composed in part (five per cent.) of Fucus crispus. From fæces containing amœbæ they claim to have cultivated the organism in association with numerous bacteria, but the morphology of the organism which they describe does not agree with that of the Amœba coli. For similar reasons it is not possible to identify with it the organisms grown by Beyerinck and by Schardinger, so that, as yet, it has not been satisfactorily demonstrated that the Amœbi coli can be cultivated on artificial media.

Pathogenicity.—The form of dysentery which is associated with the presence of the Amœba coli is endemic in certain localities, notably in Africa and India, and is often termed "tropical dysentery." It is distinguishable, according to Councilman and Lafleur, from other forms of intestinal flux by an irregular course marked by periods of intermission and exacerbation, by a tendency to chronicity, and by the frequent occurrence of abscesses of the liver. The character of the ulceration found in the large intestine is distinctive. The pathological alteration finds its chief seat in the submucosa and consists in œdema and necrotic softening of the tissues without suppuration. The mucosa is only secondarily affected and suffers in consequence of the disturbance of its nutrition produced by the destruction of the underlying submucosa. In this way is formed the typical ulcer with raised undermined edges.

The character of the stools, which are fluid and contain mucus in considerable quantity, indicate the presence of a generalized affection of the mucous membrane similar to the catarrhal inflammations of other organs. The amœbæ are usually found in greatest number in the particles of gray gelatinous material which are frequently present. They may be abundant in the mucus. Search for them is best made as soon as possible after the evacuation of the stool which should be kept warm at a temperature of about 35° C., since the movement of the organism serves to distinguish it at once from the cellular elements of the fæcal material derived from the host.

In sections of the affected intestine amœbæ are found upon the surface and in the crypts of Lieberkühn, and in the intervening tissue along which they apparently make their way, reaching after partial destruction of the muscularis mucosæ, the submucous tissue where they are found in greatest numbers. In the submucosa they are irregularly distributed, being present in the tissue which has already undergone necrosis, in that which shows only œdematous infiltration, and also in the adjacent apparently normal tissue. Bacteria are constantly associated with the amœbæ and in some cases appear to attack the tissue independently, so that, while often from the microscopical picture alone it cannot be definitely determined which is the primary invader, it is evident that, in many instances at least, the pathological changes produced are the result of the combined action of amœbæ and bacteria. As a rule, those areas in which the amœbæ are found do not exhibit leucocytic emigration, but in places suppuration is an important factor and is produced, according to Councilman and Lafleur, not by the amœbæ, but by the accompanying bacteria.

Amœbæ are present in the contents and in the walls of those hepatic abscesses which accompany the form of dysentery described

above; they are not found in the so-called idiopathic or non-dysenteric liver abscesses. On the other hand, various forms of bacteria are found in dysenteric as often as in non-dysenteric abscesses. Cultures from a certain proportion of cases of both forms remain sterile. Kartulis suggests that the amœba acts merely by transporting the pus-producing organism. It remains to be determined whether the amœba is the original invader, and if an association of organisms is usually concerned in the production of the fully formed abscess. Councilman and Lafleur state that in the smallest abscesses there is no suppuration, the lesion presenting the characteristic action of the amœba, which consists, according to their observations, in the production of necrosis of the cells and solution of the intercellular substance.

An abscess of the upper surface of the liver may after the formation of adhesions perforate the diaphragm and produce an abscess cavity in the lung, or more rarely an abscess may form in the lung, usually at the right base, though the diaphragm remains intact. Characteristic of the former condition is the expectoration of a peculiar brick-red sputum, containing amœbæ and altered liver cells.

Lösch injected fæcal material containing the Amœba coli into the rectum of four dogs, and in one of them found amœbæ eight days after the last injection. The rectum of the animal killed on the eighteenth day showed reddening, swelling, and slight superficial ulceration. Subsequently a number of observers, by injecting material from dysenteric stools containing amœbæ into the rectum of animals, have succeeded in producing an inflammatory condition. During life amœbæ have been demonstrated in the evacuations and at autopsy superficial ulceration of slight extent has been noted. The anatomical picture characteristic of the form of dysentery already described, namely, proliferation of the amœbæ in the submucosa with softening and necrosis of the tissue, has not been reproduced. The cat has been found most susceptible to such inoculations. A large number of experiments have been made upon this animal by Kruse and Pasquale, who injected portions of dysenteric stools into the rectum and prevented their escape by keeping the anus closed for from twenty-four to forty-eight hours by means of a stitch. In half of the animals death occurred spontaneously between the fourth and the sixteenth day. A hemorrhagic catarrhal inflammation of the rectum with superficial ulceration of the mucosa was observed, and in the glands of Lieberkühn and in the base of the ulcers amœbæ were found. In a considerable proportion of the affected animals active amœbæ were found in the bloody mucus discharged during life. In these

experiments a great variety of bacteria were injected along with amœbæ. To eliminate as far as possible this source of error, the contents of a dysenteric liver abscess were used instead of fæcal material and in three animals so treated results were obtained similar to those already described. In two of these successful experiments the material used was demonstrated both microscopically and by culture to be free from bacteria. Negative results were obtained from the introduction into the rectum of the stools of healthy individuals containing amœbæ, as well as by injecting therein cultures of the amœba which was found by Kruse and Pasquale to grow in unsterilized infusions of straw. Cultures of several varieties of bacteria grown from dysenteric dejections introduced in the same way gave negative results. Quincke and Roos repeated the experiments of Kruse and Pasquale, injecting into the rectum of cats, without closure of the anus, fæcal matter from a case of dysentery and obtained similar results. When material from a second case of dysentery in which amœbæ were abundant in the stools was employed the animals, nevertheless, remained healthy, no intestinal lesions being produced. These authors believe, therefore, that three varieties of parasitic amœbæ can be distinguished: (*a*) *Amœba intestini vulgaris*—nonpathogenic for man and for cats; (*b*) *Amœba coli mitis*—producing dysentery in man, but not in cats; (*c*) *Amœba coli felis*—pathogenic for both man and cats. Their distinction between the two pathogenic forms is based upon a study of only two cases and cannot be regarded as established.

The causal relationship of the Amœba coli to dysentery is by no means universally accepted, and by certain observers the organism is regarded as an accidental parasite which finds favorable conditions of growth in the diseased intestine. A great variety of bacilli and cocci have been isolated from dysenteric stools and in many instances have been regarded as the cause of the disease. Doubtless the different forms of dysentery are associated with a variety of etiological factors. In view of this diversity of opinion the important facts pointing to the pathogenicity of the organism may be summarized:

(1) Amœbæ are constantly associated with one form of dysentery, endemic in many tropical countries, and characterized by anatomical peculiarities which serve to distinguish it from the other types. The organisms are present in such numbers and so situated that the intestinal lesions may be explained by their presence.

(2) Amœbæ are found in the contents and in the walls of the hepatic and lung abscesses frequently associated with this form of dysentery.

(3) Although the anatomical picture of endemic dysentery cannot be reproduced, an inflammatory condition of the large intestine accompanied by reproduction of the organism, both within the intestinal lumen and in the affected tissues, can be produced by injecting material containing amœbæ into the rectum of animals. Of considerable importance is the fact that these results have been obtained by using the contents of liver abscesses, in which bacteria could not be demonstrated.

How do the amœbæ pass from the intestine to the liver? Four possibilities suggest themselves: (*a*) by the bile ducts; (*b*) by the lymphatics; (*c*) by the portal vein; and (*d*) by the peritoneum. The duodenum being far removed from the seat of the lesion, it is hardly probable that the organism reaches the liver along the gall duct. Although amœbæ are found in lymph spaces in the neighborhood of the intestinal lesions they are not present in the neighboring lymph glands, and it is unlikely that they are transported through a series of glands by such a circuitous route to the liver. Are they carried by the portal vessel, or do they penetrate the intestinal wall and thus reach the liver through the peritoneal cavity? Amœbæ are found at times in small vessels near the intestinal lesions. Should the organism reach the liver by way of the veins we would expect to find abscesses disseminated throughout the organ. In a large proportion of cases, however, they are situated near the surface of the right lobe in the neighborhood of the hepatic flexure of the colon or near the upper surface beneath the diaphragm. This localization makes it probable that the amœbæ wander directly from the hepatic flexure to the under surface of the liver, or entering the peritoneal cavity are carried in the direction of the lymph stream and reach its upper surface. When multiple abscesses are present in both lobes it is more probable that the organisms have been transmitted by way of the portal vein.

Amœbic abscesses of the lung are usually found in the lower lobe on the right side and in contact with the diaphragm. In many cases they represent a direct extension of a liver abscess through the diaphragm, while in others the intact diaphragm has apparently been penetrated by the amœbæ.

Other Parasitic Amœbæ.—In the uterus and in the vagina of a tuberculous patient in Tokio, who shortly before her death suffered with hæmaturia, Baelz found an amœba to which he gave the name *Amœba urogenitalis*. The organism was larger than the Amœba coli, measuring about 50 μ in diameter, but was actively motile and otherwise resembled it closely. Baelz thought it probable that it had been introduced into the vagina with water used as a douche, and had subse-

quently penetrated along the urethra into the bladder. Jürgens found at autopsy amœbæ in small cysts of the vesical mucous membrane; and in the bloody urine of an Egyptian suffering with a tumor of the bladder Kartulis found small amœbæ 12 to 20 μ in diameter. Posner observed large inactively motile amœbæ with one or several nuclei in the urine of an individual who after previous good health was attacked with a chill and subsequently voided bloody urine containing albumin and casts. At times the condition of the urine improved, the blood and albumin diminishing in amount, and at such times Posner found in addition to the motile amœbæ, which were now diminished in numbers, round bodies with granular contents and a well-marked membrane. Blood and amœbæ at times disappeared completely, though the albumin and casts persisted. The cystoscopic examination of the bladder was negative.

In the purulent contents of a large abscess communicating with the mouth Flexner found amœboid bodies. In an individual sixty years of age the removal of a small hard lump under the gum in front of the lower jaw was followed by an ulcer at the site of the operation, the bone beneath being exposed. Several months later there appeared a large abscess extending beneath the chin and over the front of the neck as far as the cricoid cartilage. In the pus evacuated at operation were found active amœbæ, with endosarc and ectosarc not readily distinguishable, but otherwise the organisms closely resembled the Amœba coli of Lösch. In the pus of a large abscess involving the submaxillary bone in an Egyptian Arab, Kartulis found very active amœbæ slightly larger than the amœba of dysentery and capable of protruding finger-like processes often exceeding in length the diameter of the body. Similar organisms were found in a fragment of necrotic bone removed at operation.

Very astonishing is the description of Leyden and Schaudinn of an amœba in ascitic fluid obtained from two patients, one a girl twenty-two years of age, suffering with an abdominal tumor, the other a man of sixty-three years, with carcinoma of the stomach. In the fluid examined at a temperature of 25° C., even when preserved aseptically for three to seven days, they found bodies 3 to 36 μ or more in diameter, which slowly altered their shape. An endosarc and ectosarc were not sharply distinguishable, though the body contained very refractive yellow granules. These authors claim that a pulsating vacuole was usually present, as well as a nucleus, and they propose the name *Leydenia gummipara* Schaudinn. L. Pfeiffer doubts the authenticity of this parasite and thinks that the structures observed by Leyden and Schaudinn were tissue cells forming part of the exudate. He states that large amœboid cells capable of independent

locomotion are found in the contents of the vesicles of smallpox, chicken-pox, herpes zoster, pemphigus, and other conditions.

II. Class: Sporozoa.

The Sporozoa form a group of organisms, all parasitic, which are characterized by their peculiar method of reproduction. The organism, usually after becoming encysted, divides into a varying, often very large, number of reproductive bodies, each of which may be enclosed in its own protective covering and is then termed a spore. The protoplasmic portion of the spore may be capable of developing into the adult organism or may again divide to form two or more sickle-shaped or amœboid bodies, each capable of repeating the life history of the parent. The group is very widely distributed, representatives being found as parasites in members of all subdivisions of the animal kingdom with the exception of the Protozoa and Cœlentrates. As a rule, each organism is parasitic in a single or in closely allied species, and passing the greater part of its life in one host, is frequently transmitted to another, while in the form of a spore. The spores are very resistant to external influences, and escaping from the body with the excretions are capable of development after residing for a considerable period of time in the water or in the soil. Reaching a suitable host in food material the spore after rupture of its capsule repeats the life history of the organism. Certain sporozoa are parasitic in two widely separated species of animals, being transmitted alternately from one to the other. The disease in cattle known as Texas fever is caused by an organism which is transmitted from one animal to another by its intermediate host the cattle tick; and recent research has rendered highly probable that the mosquito can serve as an intermediate host in transmitting the malarial parasite from one human being to another.

Sporozoa occur in a great variety of tissues and organs. In the majority of instances they exist during the whole or the greater part of their developmental stage within cells; indeed, the youngest form of all sporozoa penetrates into a cell, epithelial, muscle, blood cell, etc., and there begins its development. Having completed its intracellular growth, the organism, should it infect the epithelium of the digestive tract or kidney, escapes into the gastrointestinal canal or by the urine into the bladder, and so reaching again the external world, may be received by a new host, or it is transmitted by suctorial insects from one individual to another.

Sporozoa do not ingest solid particles of food, but obtain their nutriment by the absorption of material dissolved in the fluid of the

cell or tissue in which they exist. The parasite may cause injury to the containing cell in several ways. As a foreign body disturbing the cell structure it may cause mechanical injury; it absorbs nutritious material which would otherwise be utilized by the cell; and it may form toxic products which are harmful to its cell host.

The Sporozoa may be divided into a number of orders. Since many gaps exist in our knowledge of the group, the classification adopted by different writers is necessarily based upon insufficient data and varies greatly. The following classification may be adopted provisionally: Order: Coccidia, Gregarinæ, Hæmosporidia, Gymnosporidia, Myxosporidia, Sarcosporidia.

I. Order: Coccidia.

The Coccidia are round or oval bodies undergoing their entire development within a cell and reproducing after encapsulation by the formation of sickle-shaped reproductive bodies. They are found widely distributed among vertebrate animals and in less number among arthropods and molluscs. Birds and mammals are most frequently infected, grave diseases being at times produced by their presence. A disease often fatal, caused by the coccidium oviforme, is common in rabbits. Infection with coccidia has been rarely observed in man.

The organism always develops within an epithelial cell, usually of the gastrointestinal canal or of one of the ducts or glands communicating with it. The epithelium of the urinary and genital tracts is infected with less frequency.

The sickle-shaped bodies which represent the earliest stage of development penetrate into the epithelial cells and, assuming a round outline, develop into the adult form, a round or oval non-motile nucleated body often greatly distending the containing cell and displacing its nucleus. Preceding reproduction the body forms about itself a protective capsule. The large vesicular nucleus divides by a process of karyokinesis into numerous daughter nuclei about each of which, by division of the cell substance, an elongated rod-like body is formed. These assume a curved or falciform outline and after the rupture of the containing capsule are capable of active movement. The formation of the sickle-shaped reproductive bodies may take place by a more complicated process. The protoplasmic contents of the adult encysted organism divide into a number of nucleated bodies or sporoblasts, each of which develops a protective covering and becomes a spore. The contents of the spore subsequently divide to form sickle-shaped reproductive bodies of a number constant for a

given species. Taken into the digestive tract of a new host, the spore capsule is ruptured and the reproductive bodies are liberated. By their active movements they penetrate the epithelial cell and begin again their cycle of development. How the cells of the urinary or genital organs are affected is not known.

According to the method of reproduction the order may be divided as follows:

I. Family: Monosporidia—Forming reproductive bodies by the direct division of the contents of the encysted organism.

II. Family: Oligosporidia—Forming spores, each one of which divides to produce several sickle-shaped reproductive bodies.

Of special interest is the genus Coccidium, belonging to the Oligosporidia and characterized by the formation of four spores, each of which gives rise to two sickle-shaped bodies. Members of this genus are widely distributed and are found in mammals, birds, and fish, less frequently in amphibia and reptiles. Best known is the Coccidium oviforme of rabbits.

Coccidium oviforme, Leuckart. *Synonyms.*—Psorospermium cuniculi, Rivolta; Coccidium oviforme+Coccidium perforans, Leuckart.

The Coccidium oviforme develops in the epithelial cells of the intestine, gall bladder, and gall ducts of rabbits and gives rise to the opaque white nodules frequently seen in the liver of these animals. The adult organism is of oval form, 33 to 37 μ in length with one pole more flattened than the other, and is surrounded by a thick double-contoured capsule. The organism remains encysted while within the body of the host, and it is only after expulsion that the process of spore formation begins. The protoplasmic contents contract into a rounded mass, leaving the poles of the cyst unoccupied, and divides into four parts, each of which, at first round but subsequently oval, encloses itself within a protective capsule. The contents of the spore now divide to form two reproductive bodies of sickle shape with a knob-like enlargement at one end. The adult encysted organism occurs abundantly in the faeces of infected animals, and only after evacuation does it form spores. Mature cysts taken with food into the gastrointestinal tract of the new host set free the spores, and these in turn liberate the sickle-shaped reproductive bodies which in virtue of their active motility are able to penetrate the epithelial cells.

Rabbits from four to six weeks old confined in pens with infected animals very frequently exhibit an acute, often fatal disorder. They cease to eat and become emaciated; from the mouth and nose flows a yellow or green slimy fluid. In those that die, coccidia are found in great numbers in the epithelial cells of the gastrointestinal canal. Large areas of the epithelium are often completely destroyed. The

ingestion of a relatively small number of cysts containing four spores, each in turn giving rise to two reproductive bodies, causes an acute infection in which the intestine contains an immense number of organisms. This fact remained unexplained until L. Pfeiffer observed in animals whose intestine was the seat of fresh lesions, accompanying the adult encysted organism, round forms of slightly larger size whose contents divide directly, that is, without spore formation, into from eight to thirty-six sickle-shaped bodies. By this method of reproduction, Pfeiffer thinks, the parasite multiplies within the body of the host and gives rise to acute infections in which the gastrointestinal canal contains millions of organisms.

The motile reproductive bodies penetrate into the gall passages and infect their cells, obstructing the duct and leading to the formation of tumor-like cysts into which project papillary ingrowths covered by epithelium. Secondary changes take place in the liver substance leading to a destruction of the parenchyma and an increase of fibrous tissue in the neighborhood of the cysts.

Infection with coccidia has been rarely observed in man; the liver is the usual site of the lesion. Leuckart cites cases observed by Gubler, Dressler, Sattler, and Perls. In Gubler's case, the liver contained twenty cysts scattered through its substance. The largest, measuring 12 to 15 cm. in diameter, was palpable during life and had been diagnosed as an echinococcus cyst. It contained a thick pus-like fluid with leucocytes and numerous bodies which Gubler supposed were eggs of the *Distomum hepaticum*. Leuckart subsequently pointed out that the egg-like bodies were coccidia. Dressler found in the liver three nodules, from the size of a millet seed to that of a pea, containing soft white material in which microscopical examination demonstrated coccidia. Sattler found the parasites in the bile passages, and Perls made a similar observation in a liver which had been preserved as a museum preparation. An hepatic nodule 9–11 mm. in diameter, filled with cheesy material, was believed by Virchow to contain Pentastomum eggs, though it is possible that the tumor was due to the presence of coccidia. Podvysotski has described structures interpreted by him as coccidia in the liver cells and even in their nuclei; they occurred in four cases. From his description, however, it is not possible to identify them with coccidia.

Fatal cases of coccidial infection have been recorded by Haddon and Silcock. In an individual who had suffered with slight fever, becoming gradually unconscious before death, Haddon found white nodules upon the peritoneum, omentum, and pericardium and more sparsely scattered in the liver, spleen, and kidneys. Coccidia were present in these tumor-like nodules. A similar observation was made

by Silcock in a woman aged fifty who was suffering with an acute condition, thought to be typhoid fever. There were diarrhœa and tenderness over the liver and spleen. The liver, which was considerably enlarged, was the seat of caseous nodules resembling tubercles, but shown by microscopical examination to contain coccidia. The organisms observed in the above cases certainly resembled full-grown coccidia, but inasmuch as the process of spore formation has not been followed, it is not possible from the data furnished to identify with certainty the species concerned.

In two cases Eimer found coccidia in the epithelial cells of the intestine and several observers have encountered similar organisms in the stools. In the cases of a woman and her child both of whom were suffering with chronic diarrhœa, recorded by Railliet and Lucet, coccidia were found in the evacuations. Kjellberg found coccidia within the intestinal villi; his parasite resembles more closely the *Coccidium bigeminum* of dogs and cats than the *Coccidium oviforme* of rabbits.

Coccidial infections of more or less certain authenticity occurring in the kidney or in the kidney and ureter have been described by Lindemann, Railliet and Lucet, and Bland Sutton. Kunstler and Pitres observed a case of purulent pleurisy in which the aspirated fluid contained organisms whose identity was doubtful, though they had features in common with the coccidia. Besides numerous spindle-shaped bodies containing a nucleus were found larger round or oval cysts completely filled with similar bodies or containing in addition a central protoplasmic mass with many nuclei.

II. Order: Gregarinæ.

The Gregarinæ are parasites of round, oval, or elongated form, with an anterior end distinguishable from the posterior, but symmetrical as regards their long axis. Many forms are divided by transversely situated partitions into two or three segments, one behind the other. They undergo part of their development within cells and after encapsulation reproduce by spore formation. The Gregarinæ form a very interesting biological group widely distributed among invertebrate animals. They are very frequently parasitic in Arthropods, particularly in centipedes, cockroaches, and cray-fish. They have never been found in vertebrates.

The earliest phases of development take place within epithelial cells, usually those of the gastrointestinal canal. As the size increases the organism can no longer be contained in the infected cell and hence escapes into the lumen of the canal, where it may persist as a motile

body or attach itself to the wall. In certain cases, as it grows, it penetrates in the opposite direction and instead of entering the intestine forms a projection into the peritoneal cavity and finally may become free therein.

Many gregarinæ are divided into three sections, the divisions being marked upon the surface by ring-like grooves. The anterior segment is of smaller size than the others and varies greatly in shape, being frequently pointed or provided with hooks and serving to fasten the organism to some object. It is not an essential part of the cell body, being frequently detached by accident and always lost before the encystment preceding sporulation. The middle and posterior sections forming the bulk of the organism are separated by a transverse partition. The nucleus is situated in the posterior compartment. Gregarinæ divided into three sections are spoken of as Tricystidæ. Another group of gregarinæ, the Bicystidæ, want the partition separating the posterior and middle sections, so that the organism consists of a smaller anterior portion, whose function is to attach the body to some object, and a posterior section containing a nucleus. Other forms, the Monocystidæ, show no separation into two or three portions, but consist of an undivided body, one end of which usually differs in shape from the other, and is always directed forward when the organism moves.

In the protoplasm of the cell body are distinguishable an internal granular endosarc and an external clearer zone, the ectosarc, the outermost portion of which forms a protective cuticle. In the ectoplasm are contractile fibres by means of which movements are produced. These consist of a simple bending of the cell body with little or no alteration of position, or an active progressive movement with no evident alteration of shape, the so-called gregarine movement, in explanation of which no satisfactory theory has been advanced.

Before sporulation takes place the organism throws off its anterior section, assuming a round or oval form, and encloses itself in a firm resistant membrane. The encapsulated forms of the Dicystidiæ and Tricystidiæ escape from the host before spore formation begins; those of the Monocystidiæ mature within the body cavity into which they project, at times becoming free within it. The nucleus by a process of karyokinesis forms a large number of daughter nuclei, and the protoplasm also dividing, forms about each a round body. These bodies surround themselves with a protective capsule and are set free by the rupture of the containing cyst. The contents of each spore divide into six to eight nucleated reproductive bodies or sporozoites, elongated structures, pointed at both ends. The spores taken into the digestive tract of a suitable host set free the reproductive bodies,

each one of which by its active motion is able to penetrate an epithelial cell, and assuming a round or oval shape may finally develop into the three-chambered form of the adult.

III. Order: Hæmosporidia.

The Hæmosporidia are gregarine-like sporozoa of elongated form developing in the blood corpuscles and multiplying by division within a capsule into a number of reproductive bodies. They may, during a portion of their life, exist free in the plasma, but reproduction takes place within a cell of the blood or of one of the blood-forming organs. Hæmosporidia unlike the Gregarinæ are found only in vertebrate animals. First observed in frogs by Ray Lankester in 1871, they have since been found in lizards and turtles. Their development takes place within the red blood corpuscles, occasionally, it is said, in the leucocytes, and only the adult forms are capable of life when free in the blood plasma. The infected corpuscles lose their hæmoglobin and undergo degenerative changes; but what effect the parasite has upon its host is not known. In structure the Hæmosporidia resemble those Gregarinæ which are not divided into sections, the Monocystidiæ, and have worm-like bodies pointed at one or both ends. The parasite is usually about as long as the containing corpuscle, but at times it is twice this length, being bent upon itself. A hyaline ectosarc is, as in Gregarina, distinguishable from a granular endosarc. The nucleus, consisting of chromatin particles and achromatinic nuclear substance, is usually separated from the cell body by a nuclear membrane. In certain forms, for example, in the *Drepanidium princeps* of the frog, there is no nuclear membrane, and the nucleus consists of a clear zone surrounding one or more chromatin particles, thus resembling the much-discussed nucleus of the human malarial parasite and allied forms. During its extracellular existence the organism is capable of very active movements which resemble those of the Gregarinæ. Progression may be effected by a snake-like gliding without evident alteration of form, or by wave-like contractions passing from the anterior to the posterior ends of the cell body. Such movement may be so active that the organism penetrates into and through red corpuscles and leucocytes in its way.

The reproduction of the Hæmosporidia always takes place within a cell, usually a red blood corpuscle. Labbé has observed with *Drepanidium princeps* of frogs and with *Karyolysus lacertarum* of lizards, conjugation of the motile organisms. He thinks that the adult parasite, having escaped from the corpuscle in which it has developed after existing for a time free in the plasma, enters a second time a red

blood corpuscle and there undergoes the reproductive process. The organism assumes a rounded outline, and from the ectosarc is formed a membrane or cuticle enclosing the body. These encysted forms are usually found in the spleen, bone-marrow, or liver, rarely in the circulating blood. The nucleus divides by karyokinesis into a large but variable number of parts. The protoplasm separates into segments, each of which contains one of the particles of nuclear substance, so that finally there are formed a number of small oval or sickle-shaped reproductive bodies, ultimately set free by the disintegration of the containing corpuscle and the rupture of the cyst. The reproductive body penetrates a corpuscle and develops into the adult form, which, escaping, is capable of extracellular, motile existence. How the parasite is transferred from one host to another is not known.

IV. Order: Gymnosporidia.

The Gymnosporidia, according to the classification of Labbé, are parasitic amœboid Sporozoa reproducing, without any preceding encapsulation, by dividing into a variable number of amœboid or sickle-shaped bodies. The members of this order are found only in vertebrates and, with the exception of one group parasitic in the intestinal epithelial cells of frogs and salamanders, develop exclusively in the red blood corpuscles. Such parasites are spoken of as Hæmocytozoa. The most important members of this group are the organisms which cause the malarial fevers in man. Closely allied hæmocytozoa, however, frequently infect birds. It is probable that the organism causing Texas fever in cattle belongs to this order, although as yet, its life history being very imperfectly understood, it cannot be classified with certainty.

The Gymnosporidia resemble in appearance amœbæ, being naked protoplasmic bodies in many instances capable of changing their form by the protrusion of pseudopodia. Reproduction takes place by the division of the cell body into a number of segments, each of which, unprovided with a protective covering, enters a red corpuscle and repeats the cycle of development. The species of Gymnosporidia inhabiting the epithelial cells of the intestine of frogs and salamanders form sickle-shaped reproductive bodies and are for this reason separated by Labbé, to constitute the family Acystidæ, from the remaining members of the order included in the family Hæmamœboidæ. The latter segment to form amœboid reproductive bodies and include the malarial parasites of man and the closely allied species of birds.

The study of the hæmocytozoa of birds, interesting in itself, has recently thrown much light upon certain problems concerning the parasites of the malarial fevers. Danilewsky (1885) observed in the

nucleated red corpuscles of birds clear bodies lying on one side of the nucleus and curving over its ends, thus having a crescentic or so-called halter-shape. Grassi and Feletti (1890) subsequently described a second form of organism of smaller size and irregular shape lying at one end of the corpuscle and displacing the nucleus to the other. These two types have been observed in a great variety of avian species, sparrows, pigeons, owls, crows, etc., and in widely separated regions, Europe, America, and India. To the first form Labbé has given the specific name *Halteridium danilewski*, to the second, *Proteosoma grassi*. These organisms resemble the malarial parasites of man and differ from other hæmocytozoa in two important characteristics: (*a*) They form brown pigment from the hæmoglobin of the containing corpuscle; (*b*) under certain conditions actively motile flagella-like structures are formed.

The frequency of hæmocytozoan infection varies in different localities. In parts of Italy almost all birds are infected, while in northern France, of sixty-six species examined by Labbé, members of only four were found to contain intracorpuscular parasites. In the United States, in the neighborhood of Baltimore, and in Canada, near Toronto, hæmocytozoa have been shown to occur in birds with considerable frequency. But few observers have noted any marked disturbance of the birds' health as a result of their presence. Danilewsky has, however, attempted to demonstrate the existence of symptoms analogous to those of the malarial fevers. He describes an acute infection caused by the irregularly shaped parasite and manifested by elevated temperature and loss of weight; and a chronic form caused by the halter-shaped organism and usually unaccompanied by evident symptoms. Doubtless, however, the blood may contain either form in considerable number without any noticeable disturbance of the infected bird.

Proteosoma Grassi, Labbé.—The parasite undergoes its entire cycle of development within the red blood corpuscle, the youngest form appearing within the corpuscle as an approximately round refractive body, resembling a small vacuole. As it grows it acquires black pigment granules, formed from the hæmoglobin surrounding it. The larger pigmented bodies vary considerably in shape, and though they often present short blunt processes, amœboid movements are not evident. The pigment, in the form of fine black granules, is usually collected into a loose clump, situated near the periphery of the body. The growing organism, at first located in any part of the corpuscle, comes to occupy one extremity, the nucleus being displaced from its normal situation into the other end, usually assuming a position with its long axis nearly at right angles to that

of the corpuscle. All phases in the process of reproduction may be observed in specimens of blood freshly drawn from the peripheral circulation. The parasite assumes an approximately round outline, the pigment being collected into a solid mass in the centre. Indentations at regular intervals appear about the margin and give the body somewhat the appearance of a rosette. Situated near the periphery is a circle of minute refractive dots, each one midway between two of the indentations. These indentations, extending towards the centre as radially arranged striations, become more marked and divide the organism into a variable number (roughly speaking, five to twenty) of small bodies which assume a circular outline. The stroma of the containing corpuscle is often somewhat decolorized, and the nucleus is crowded into one end. Finally, there is a dissolution of the rim of the corpuscle still remaining, and the segments are set free in the blood plasma ready to enter again red corpuscles.

The use of certain stains, notably methylene blue, gives us information concerning the internal structure of the parasite. The small non-pigmented bodies stain at the periphery, the central portion remaining almost completely uncolored. Within can be demonstrated, by special methods of staining, particles of chromatin. In the larger forms the whole body takes a fairly deep stain with methylene blue and assumes a granular appearance. The segmenting bodies correspond in size to the largest of the full-grown forms and stain deeply; the chromatin divides into a variable number of parts, one for each segment formed. When the process of segmentation has almost reached completion the body consists of a number of approximately round, clear areas surrounded by more deeply stained protoplasm, while within the clear spaces may be seen minute specks whose staining-reaction shows them to be chromatin; they correspond to the refractive dots of the fresh body.

In addition to the full-grown organism corresponding in size to the segmenting bodies there are found much larger forms distending the containing corpuscle and at times causing the extrusion of its nucleus. They are less refractive than the more common smaller bodies and in stained specimens remain almost completely uncolored. They are apparently not destined to segment, but, as will be seen, undergo a process known as flagellation.

Halteridium Danilewsky, Labbé.—The youngest forms resemble those of the proteosoma. The organism as it grows becomes drawn out, and pigment granules are seen scattered in its substance. It lies, as an elongated body, along one side of the nucleus of the containing corpuscle and, as it increases in size, curves around the two

ends of the nucleus, thus assuming a crescentic or halter-shaped outline. The protoplasm of these bodies has a somewhat granular appearance and takes a deep blue stain with methylene blue. A sharply defined, approximately round area remaining unstained stands out prominently. This area contains chromatin particles which may be demonstrated by special methods of staining, best by that of Romanowsky, and represents apparently the nucleus of the organism.

Besides these deeply staining forms, at times in almost equal abundance, as pointed out by Opie, are full-grown bodies remaining almost completely unstained, analogous with the faintly staining bodies of proteosoma. The middle portion of the organism is an almost untinged oval space surrounded by a rim of faintly stained material containing the pigment granules and thickest at the poles, where all the pigment is frequently collected. It is these bodies which undergo flagellation.

Segmentation is not observed in the circulating blood. Labbé has found segmenting bodies in the bone-marrow and spleen of the lark. According to him, the nucleus of the parasite divides to form two daughter nuclei, each of which, passing to one extremity of the organism, divides into from six to fifteen segments.

Flagellation.—After the blood is drawn certain of the above-described organisms undergo a process spoken of as flagellation and characterized by the formation of long, actively motile filaments or flagella. The process is in all essential details the same in both proteosoma and halteridium, but can be studied with the greater facility in the latter. If a fresh specimen of blood is prepared and rapidly brought under the microscope, full-grown bodies lying curved along one side of the nucleus of the corpuscle are seen to collect themselves into an oval, then into a circular form. The rim of the corpuscle surrounding the parasite disappears, apparently absorbed in the plasma, and a round extracellular body remains lying beside the nucleus of the corpuscle which formerly contained it. Very soon the extracellular body acquires an active vibratory motion as the result of the presence of from two to four or even more active flagella, which have suddenly made their appearance at its periphery. After a time individual flagella are not infrequently seen to become detached and float away continuing their active serpentine movements. A very remarkable phenomenon has been observed by MacCallum in the halteridium. According to his observations, only the palely staining non-granular bodies form flagella. The flagella break away from the parent body and seek the more granular forms which are characterized by their greater ability to take up staining-substances. Several may collect about such an organism and beat against its body with an active

wriggling motion. One finally projects itself into the organism and is lost, merged in its substance. The granular bodies are, he thinks, to be regarded as female forms, the non-granular as male; the flagella have a spermatozoa-like action. After fertilization the body remains quiet for from fifteen to twenty minutes. At the end of this time a conical process appears at one point and the body assumes an elongated form with one end bluntly pointed, the other having attached to it a spherical appendage containing most of the pigment. The organism is now capable of very active progressive movement, which takes place in the direction of the pointed end and is apparently the result of waves of contraction passing from the anterior to the posterior extremity. The movement is of such force that red corpuscles or leucocytes in its path do not obstruct its progress, but are shattered by contact with the pointed end.

Method of Infection.—Conflicting statements are made concerning the possibility of transmitting hæmocytozoan infection from one bird to another by intravenous or subcutaneous inoculation. Di Mattei in a large number of carefully conducted experiments obtained negative results. Recent investigations made by Ross in India prove that the mosquito may act as an intermediate host in the transference of infection from one bird to another. His first observations concerned the development of the proteosoma within the body of mosquitos which had bitten birds infected with the organism. Examining mosquitos which had ingested proteosoma-containing blood two days before, he found in or adjacent to the muscle layer of the insect's stomach sharply outlined oval bodies 6 to 7 μ in diameter, containing pigment granules resembling those found in the intravascular parasites. These pigmented bodies were observed in the stomach wall of mosquitos fed upon the blood of birds containing proteosoma, while they were absent after the ingestion of normal blood or of blood infected with halteridium. By examining mosquitos at varying intervals after feeding it was found that these bodies gradually increase in size, having by the third or fourth day increased their diameter three or four times. The protoplasm becomes granular and vacuoles and oil-like droplets appear. The pigment granules diminish in size and number, and finally disappear. By the fourth or fifth day, having assumed a spherical form, these bodies are 60 to 70 μ in diameter and have acquired a refractive double outline, evidently representing a resistant capsule. They now project as wart-like protrusions from the external surface of the stomach into the body cavity of the insect. About the eighth or ninth day these encapsulated structures may rupture and set free in the body cavity great numbers of minute, somewhat flattened, spindle-shaped bodies 12 to 16 μ in

length and about 1 μ in breadth. These have no motility, but are carried in the body fluids to the various tissues of the insect.

On examining the tissues in the neck of the mosquito, Ross has found a gland, apparently overlooked by zoologists, composed of cells arranged about a branching duct which opens at the extremity of the proboscis. He found within the cells of this gland in mosquitos fed about ten days before upon the blood of birds infected with proteosoma the spindle-shaped bodies which he calls germinal rods. From this gland he thinks an irritant material is injected into the wound made by the insect, so that the spindle-shaped bodies are now in a favorable position for transmission into a new host.

These observations strongly suggested a possible method of transmission, and Ross, continuing his experiments, attempted to transfer proteosoma from one bird to another by the mediation of mosquitos. He allowed the insects to feed upon the blood of an infected bird, and after keeping them for over a week, in order that the spindle-shaped bodies might have time to develop, caused them to bite birds previously found to be uninfected. His results are convincing. Of twenty-eight sparrows so treated, twenty-two became infected. He was also able to infect a crow and four weaver birds with mosquitos fed upon the blood of an infected sparrow.

The Parasite of the Malarial Fevers. *Synonyms.*—Oscillaria malariæ, Laveran; Hæmatozoon malariæ, Laveran; Plasmodium malariæ, Marchiafava and Celli: Hæmatophyllum malariæ, Metchnikoff; Hæmatomonas malariæ, Osler; Hæmamœba laverani, Labbé.

The discovery of a protozoan microorganism whose development within the blood causes malarial fever was made in 1880 by Laveran, a French military surgeon stationed in Algiers. The existence of colorless bodies containing granules of pigment in the blood of individuals suffering with the malarial fever had long been known; indeed, the description which Meckel gives of them in 1847 leaves no doubt but that he saw what has since been proven to be animal parasites. While studying the pigment in the blood of a patient with malaria, Laveran observed a pigmented body having at its periphery motile filaments whose great activity suggested at once a parasitic organism. He subsequently described crescentic and ovoid bodies containing pigment and smaller transparent non-pigmented bodies of spherical shape, attached, as he believed, to the surface of the red blood corpuscles. Ricard confirmed these observations, but pointed out that the parasite in its earliest stage, a minute clear body without pigment granules, grows in a corpuscle, and not upon it as Laveran believed. For several years the publications of Laveran attracted but little attention. Marchiafava and Celli studied the bodies which he

had described, and came to the conclusion that they represented degenerative changes in the red blood corpuscles. In 1885, however, observing amœboid movements of the intracorpuscular bodies, they became convinced of their parasitic nature and proposed for them the name of Plasmodium malariæ.

Important considerations relating to the development of the parasite and the type of organism present on the one hand, and the clinical phenomena of the disease on the other, have been pointed out by Golgi. Studying particularly the regularly intermittent fevers of the tertian and quartan type, he observed that the organism develops in groups of which all the members are at the same time in the same phase of development. The malarial paroxysm is coincident with the reproduction by segmentation of the organisms composing such a group. He observed that the parasite associated with quartan fever presents peculiarities which serve to distinguish it from that of the tertian type, while with neither could he demonstrate the crescentic bodies described by Laveran. The cycle of development of the quartan parasite, he found, has a duration of seventy-two hours, while the tertian organism presenting distinguishing morphological points develops in forty-eight; and upon this divergence of interval depends the distinction between the two clinical types of the regularly intermittent fevers. Differential points between the tertian and quartan parasites on the one hand, and that associated with the irregular estivoautumnal fever on the other hand, were apparent. The observations of Laveran and Golgi have been confirmed by a very large number of observers in Italy, Austria, Russia, India, Africa, and America. Councilman, Osler, Dock, Thayer, and Hewetson have shown that the malarial infections of this country are caused by the three types of organisms described by the French and Italian observers.

For the purpose of diagnosis the malarial parasite is most readily observed in specimens of freshly drawn blood examined while the organism is still alive. For an hour or more such preparations may be satisfactorily examined and the morphology and vital phenomena of the organism observed. Additional information concerning its structure is obtained by the study of dried blood films fixed by heat or other means and subsequently stained. The method of staining which has been most serviceable is that introduced by Romanowsky, and subsequently modified by a number of observers. Specimens are stained in a mixture of eosin and methylene blue or, as proposed by Nocht, eosin and polychrome methylene blue. The body of the organism stains blue, while its chromatin substance takes a lilac color. A convenient method of rapidly staining the parasite for diagnosis has been proposed by Futcher and Lazear. Smear preparations are

immersed for one minute in a one-fourth-per-cent. solution of formalin in ninety-five-per-cent. alcohol. The specimens are washed in water and then treated for from ten to fifteen minutes with a stain composed of one part of saturated solution of thionin in fifty-per-cent. alcohol and five parts of a two-per-cent. carbolic-acid solution. They are mounted in Canada balsam.

Species of Parasites.—Golgi studying the regularly intermittent fever was able to demonstrate that the quartan type, with paroxysms recurring every fourth day, was associated with the presence of a protozoan microorganism which differs, morphologically and biologically, from that observed with the tertian type. And it soon became evident that the organism causing the more severe malarial infections with irregular febrile course differs markedly from the above-mentioned parasites.

While the great majority of observers recognize the existence of three readily distinguishable parasites, a considerable number, including Laveran, believe that they do not represent distinct species, but are varieties of a single polymorphic organism, which under varying conditions may assume various forms. They suggest that a change in season influences the structure of the parasite. The impossibility of cultivating the organism outside of the body deprives us of a very important method of determining its specific identity. It has, however, been found possible to transfer malarial infection from one individual to another by the subcutaneous or intravenous injection of infected blood. In all such experiments, when performed with the necessary precautions, the same variety of parasite as that injected has been found in the blood of the inoculated individual and the type of fever produced has corresponded to that affecting the patient from whom the infection was transferred. Moreover, prolonged observation of patients infected with one or other form of parasite has failed to disclose its transformation into another variety. In fine, the weight of evidence is at present in favor of a specific individuality of the three forms mentioned. For a description of the different species of malarial parasites the reader is referred to the article on Malaria in this volume.

Flagellation.—Various opinions have been advanced as to the nature of the flagellate bodies which differ but little in the three species of malarial parasite. The phenomenon of flagella formation occurs after the blood has been drawn and subjected to the consequent chemical and physical changes. A number of observers have therefore come to the conclusion that it represents a degenerative or agonal process preceding the death of the organism. The structure and activity of the flagella on the other hand have led many to believe

that the process is related in some way to the extracorporeal existence of the organism. The process exhibited by the malarial parasite does not differ from that observed in the gymnosporidia of birds; the bodies which develop flagella here as in the bird differ from those undergoing segmentation, in the tertian and quartan parasite being very large forms which stain palely, in the estivoautumnal organism the crescents or ovoid bodies. The observations of MacCallum indicate that in the bird the process represents a method of fertilization, the free flagellum acting as the male element. By analogy it seems not improbable that the same process takes place in man. Again MacCallum in a case of estivoautumnal fever has seen a flagellum free itself from a round extracellular body and penetrate a second organism, derived like the first from a crescentic form, but differing in having its pigment collected into a central ring. Nothing corresponding to the pseudovermicules of the bird has been observed.

Method of Infection.—Numerous experiments by Gualdi and Antolisei, Di Mattei, Elting, and others have demonstrated that malarial fever can be transmitted from one individual to another by both intravenous and subcutaneous injection of blood containing malarial parasites. In every instance in which the inoculated blood has been carefully studied that variety of organism with which the malarial patient supplying the blood was infected has been found in the inoculated individual and the corresponding clinical type of fever has been reproduced. Elting has recently shown that blood containing only crescentic and ovoid bodies does not reproduce the disease. He inoculated with uniform success six individuals with blood containing amœboid estivoautumnal parasites, but in three instances in which blood containing only crescents and ovoid bodies was intravenously injected, negative results were obtained.

Until recently no theory concerning the life of the malarial parasite outside of the human body has been based upon any satisfactory data. The experimental investigations of Ross upon the Proteosoma of the bird pointed very strongly to the possibility that the mosquito may act as an intermediate host for the malarial parasite. That these results may be used in the explanation of human infections is indicated by certain additional facts. Ross has found in the stomach wall of mosquitos fed upon the blood of individuals infected with estivoautumnal or tertian malaria pigmented cells resembling those found after the ingestion of the blood of infected birds; and Bignami, Bastianelli, and Grassi have confirmed the observations of Ross, finding similar pigmented cells gradually developing in the stomach wall of mosquitos of the species Anopheles claviger fed upon blood containing crescentic organisms.

Apiosoma Bigeminum, Smith. *Synonym.*—Pyrosoma bigeminum, Smith.

The disease of cattle known as Texas fever is caused by an intracorpuscular parasite which may be, provisionally at least, included among the gymnosporidia. The disease, which has a wide geographical distribution, being found in the Southern States and in parts of Europe and Africa, may assume an acute, usually fatal form, running its course in three or four days and characterized by fever, rapid destruction of the red blood corpuscles, hæmaturia, and, at autopsy, great enlargement of the spleen. A much milder chronic type, often unaccompanied by marked symptoms, is associated with a gradual diminution of the number of red blood corpuscles.

The microorganism of Texas fever was discovered by Theobald Smith, who called it Apiosoma bigeminum. In the red blood corpuscles of animals affected with the disease are found homogeneous pale bodies of a pyriform outline, one end being round, the other tapering to a point. Usually two such bodies are found in the same corpuscle; they lie with their pointed ends close together, but not apparently in continuity. Similar forms, though lacking the pear-shaped outline, occurring singly in the corpuscle, exhibit active amœboid movements. A nucleus is not evident in fresh specimens, though in the rounded end of the pear-shaped bodies a refractive spot is often seen; Ziemann has demonstrated the presence of chromatin particles having the staining-reactions of those of the malarial parasites. Intracorpuscular organisms are found in much greater number in the blood of the liver, kidney, and heart muscle than in that of the peripheral circulation. In the chronic form of Texas fever the above-described organisms are not found, but minute coccus-like bodies, often in pairs, are present in the corpuscles. The method by which the organism reproduces is not understood, nor has the relation of the larger forms to the coccus-like bodies been demonstrated.

The researches of Smith and Kilborne have shown that the disease is transmitted by the cattle tick—*Boophilus bovis*. The adult insect, after living upon the skin of an infected animal upon whose blood it feeds, drops to the ground and lays its eggs. The larvæ developing from these eggs, attaching themselves to another animal, are capable of transferring the disease. Infected animals do not infect others if the ticks are carefully destroyed, and numerous experiments have shown that animals may be infected by larvæ hatched artificially from the eggs of ticks taken from diseased animals. These observations have recently been confirmed by the work of Koch in Africa.

V. Order: Myxosporidia.

The Myxosporidia are sporozoa possessing an amœboid protoplasmic body containing a number of nuclei and forming spores by a process which begins at a very early stage of development. The members of this group are parasitic in a variety of invertebrate animals. Among vertebrates they are common in fishes (psorosperms of fishes) and give rise to tumor-like formations injurious to the host. Widespread fatal epidemics due to myxosporidia not infrequently occur. The organism in many cases lives within the cavities of hollow organs, as the gall-bladder or the urinary bladder, and is found swimming freely or attached to the epithelial cells, while other forms occur in the tissues and give rise to cysts, which are often of considerable size. Again, they may penetrate into the tissues and, becoming diffusely scattered, may produce an inflammatory reaction. Many of the myxosporidia, unlike other sporozoa, do not develop within cells, but lie free in the tissues, while in certain cases development begins within a cell and is completed after destruction of the cell host.

The method of reproduction of the myxosporidia is characteristic. Spore formation may take place within the cell body of the microorganism without cessation of its movements and growth. The process begins with the formation of a globular mass of protoplasm containing one of the many nuclei present. This surrounds itself by a delicate membrane and within are formed usually two spores, which are more or less regularly oval in outline and are provided with from one to four small cyst-like structures opening upon the surface at one or both poles. These so-called polar capsules contain a long, delicate filament which can be thrust forth. The spore enters the digestive tract of a new host and by the rupture of its capsule liberates an amœboid body.

The order contains a great variety of forms. Gurley distinguishes one hundred and two species in the fish.

The parasite causing the pebrine disease of silk worms belongs to this group. The ravages of this microorganism, *Glugea bombycis*, Théolan, are indicated by the statement of De Quatrefages, that the loss suffered by France in the thirteen years following 1853 reached a thousand million francs, while in Italy, where the disease shortly after showed itself, the loss was about twice that amount. The spores of the parasite, oval or pear-shaped refractive bodies, surrounded by a capsule, are ingested by the silk worm. The contents escaping from the capsule as amœboid bodies infect the intestinal epithelium and

the muscularis as well; increasing in size, as with other myxosporidia, spores are formed within the protoplasmic body and finally become free in the tissues. The parasite is distributed in great numbers throughout the body, and even the egg of the adult moth is infected; but since the ability of the latter to develop is not lost, the disease is transmitted to a new generation. As a result of the investigations of Pasteur the ravages of the disease have been checked; the silkworms or the eggs are examined microscopically, and only those found to be healthy are used to produce a new stock.

An organism producing lesions of the skin and internal organs resembling those of tuberculosis described by Rixford and Gilchrist is believed by Stiles to present closer analogies to the myxosporidia than to other orders.

Coccidiodes Immitis, Rixford and Gilchrist.—Rixford and Gilchrist have reported two cases of infection with a protozoan microorganism to which they give the name Coccidiodes immitis. The disease occurring in California began as a lesion of the skin which clinically resembled tuberculosis. Subsequently, neighboring lymph glands became involved and death resulted in both cases. An autopsy performed upon one of the cases disclosed nodules resembling tubercles in the lung and spleen and upon the peritoneum. The various lesions found differed microscopically from those of tuberculosis only in the absence of tubercle bacilli, while protozoan microorganisms were present in great number, at times free in the tissue, at other times within giant cells. They were numerous in the pus from the skin lesions.

The full-grown organism is a spherical body 15 to 27 μ in diameter enclosed in a conspicuous double-contoured capsule. A nucleus has not been demonstrated. Reproduction takes place by repeated division of the protoplasmic contents so that a very large number of small bodies are formed. These set free by the rupture of the cyst gradually acquire a capsule and repeat the process of reproduction. By what method they are transferred from one host to another is not known.

The protozoan microorganism previously found by Wernicke in similar skin lesions in a case occurring in Buenos Ayres very closely resembles that described by Rixford and Gilchrist. This parasite is an encapsulated body existing free in the tissues and within the giant cells. Nevertheless, from the description which Wernicke gives, it cannot with certainty be identified with the Coccidiodes immitis, though it seems probable that he was dealing with the same microorganism.

VI. Order: Sarcosporidia.

The Sarcosporidia are oval or elongated organisms parasitic within the muscle fibres and reproducing by means of kidney-shaped or falciform bodies.

They have been observed only in vertebrates, being present with great frequency in sheep, pigs, and other domestic animals, while several cases of sarcosporidian infection of man are described. The parasite at first develops within the muscle fibres, but later in the course of the infection may, like the myxosporidia, give rise to cysts or to a diffuse infiltration of the tissues. In general its pathogenic significance is slight.

The parasite occurs within the muscle fibre as an elongated tubular body with somewhat pointed ends, the so-called Miescher's or Rainey's tube. The full-grown forms are provided with a cuticle composed of two layers, an outer, thick and radially striated, and an inner, thinner and homogeneous. Within the cuticle of even the youngest forms are seen a number of round corpuscles, each containing a nucleus. As they increase in number those more centrally situated, acquiring an enclosing membrane, divide to form kidney or sickle-shaped reproductive bodies. By what method the sarcosporidia pass from one host to another or how they reach the muscle fibres is not known; presumably they enter by the digestive tract and hence migrate to the muscle.

Sarcosporidia occurring in man have been described by several observers. Lindemann and Rosenberg have recorded cases in which bodies resembling sarcosporidia formed tumor-like structures in the substance of the heart or upon its valves, but from the description which they have given it is by no means certain that they were dealing with genuine sarcosporidia. Kartulis has described a case of multiple abscesses of the liver and of the abdominal wall in a Sudanese. Within the wall of the large liver abscesses twisted tubules surrounded by a cuticle and containing numerous round corpuscles were observed lying in the connective tissue. In the wall of the muscle abscess were oval and round structures filled with round or sickle-shaped bodies and in the interfibrillar tissue of the muscle were seen large cysts or Miescher's tubules containing round, oval, and sickle-shaped bodies enclosed within a thick cuticle. In the laryngeal muscle of a man, Barbaran and Saint-Remy found sarcosporidia within the muscle fibres. These parasites were typical in form and belong, according to the authors, to the species *Miescheria muris* frequently seen in the muscles of domestic animals, as cattle and sheep.

III. Class: Mastigophora.

The Mastigophora include a very large number of organisms of great diversity of structure, whose common characteristic is the possession of one or more flagella. The organisms of this group having a parasitic existence are few in number and belong to the order Flagellata, whose members are distinguished by the presence of one or more flagella situated upon the anterior end. The cell body is usually oval or pear-shaped, rarely more spherical or much elongated. An anterior end is distinguishable, the flagella being directed forward in locomotion. The lateral situation of an orifice into which food is taken or of an additional flagellum or of some other structure distinguishes a ventral from a dorsal surface. Certain forms possess in addition to flagella a motile organ, the so-called undulatory membrane, consisting of a sheet of protoplasm attached along the surface of the cell body and capable of wave-like movements.

Certain members of the groups are provided with a mouth and can ingest solid particles; others, including the parasitic forms, obtain their nutriment by absorbing soluble material from the fluid medium about them. Reproduction usually takes place by simple binary fission or by the formation after the conjugation of two individuals of a large number of reproductive bodies within a capsule. In certain instances the two individuals which take part in the process of conjugation are unlike and may be distinguished as male and female elements.

Parasitic flagellata are occasionally found in the hollow organs, the intestine, vagina, or urinary bladder of vertebrates. Others are parasitic in the blood plasma. Grassi recognizes among the flagellata five families each of which includes parasitic species. His classification, as will be pointed out, is defective in certain details, but forms a convenient method of grouping.

I. Family: Cercomonas.

The group is, according to Grassi, composed of organisms whose posterior extremity is narrow and tapering, at times bifid; several flagella are attached to the anterior end. Certain flagellate organisms found in the human bladder and intestine have been classified in this family; under the name *Bobo urinarius* Kunstler has described an organism with an anterior rounded end, to which are attached two flagella, and a posterior tapering extremity. He demonstrated it in the freshly passed urine of a patient with pyelitis following cystotomy.

In the discharges of two patients, one suffering with typhoid fever, the other with cholera, Davaine (1854) discovered an organism of similar shape provided with but one flagellum and gave to it the name *Cercomonas hominis*. In one hundred cases of diarrhœa Grassi found similar organisms to which he gave the name *Monocercomonas hominis*, placing them in the family Cercomonas. At the anterior end, according to his description, are three or four flagella, one of which is directed backward. The parasite, however, belongs to the family Trichomonadidea, since it is evident that Grassi mistook the undulatory membrane characteristic of this group for a flagellum directed backward. It is, moreover, probable that many parasites which have been believed to belong to the family Cercomonas are in reality trichomonads, the details of their structure having been incorrectly observed or interpreted and the existence of organisms having the characteristics assigned by Grassi to the cercomonads is doubtful.

Flagellate organisms described as cercomonads were found by Kannenberg in the sputum of two individuals suffering with gangrene of the lung and in one case were found at autopsy in the gangrenous cavity. Streng has made a similar observation in three cases. Litten described cercomonads in the pleural exudate of a case of pneumothorax, and Roos has, he thinks, observed them in the exudate of a putrid pleurisy caused by the perforation of a lung cavity.

II. Family: Lophomonadidea.

The members of this group closely resemble the cercomonads, according to Grassi, both in form and in the possession of flagella attached to the rounded end. The distinctive feature, he states, lies in the number of flagella, which here is large; while with the cercomonads it does not exceed three or four. No parasites of present interest belong to the family.

III. Family: Megastomidea.

As defined by Grassi, the family contains organisms characterized by a bifid posterior end and a deep concavity upon the ventral surface near the anterior end. To this group belongs an organism, the Megastoma entericum, found in the intestine of man and of certain lower animals.

Megastoma Entericum, Grassi. *Synonyms.*—Cercomonas intestinalis, Lambl; Hexamita duodenalis, Davaine; Dimorphus muris, Grassi; Megastoma intestinale, Blanchard; Lamblia intestinalis, Blanchard.

The parasite was first observed by Lambl (1859) in the mucous discharges of a child suffering with diarrhœa; he identified it with that described by Davaine and gave it the name Cercomonas intestinalis, but it is evident from his description that he was dealing with a different organism. Grassi subsequently described the same parasite and gave it the name Megastoma entericum. It is parasitic in the intestine of the mouse, rat, rabbit, cat, dog, sheep, and man, and is usually found in the duodenum and jejunum.

The organism, surrounded by a delicate cuticle, is pear-shaped, its posterior end tapering to a point. Near the anterior end, upon the ventral surface, occupying about two-fifths of the length of the body, is a deep excavation, kidney-shaped in outline, with a well-defined margin which posteriorly forms a slight projection. By means of this excavation the body attaches itself to the surface of an epithelial cell. Four pair of flagella are present; one arises from the lateral margins of the excavation, two from the projection of its posterior margin, the fourth from the posterior extremity. At the bottom of the excavation within the cell are two vesicular structures united by a narrow band and believed to represent a nucleus. The parasite assuming an oval form occasionally appears in the dejections as an encapsulated body. Doubtless human beings are infected by ingesting cysts, evacuated by the mouse or other animal.

Although the Megastoma entericum is not infrequently found in the stools of individuals suffering with diarrhœa, there is no evidence that it bears an etiological relation to the pathological process. In animals it occurs in the normal intestine. Müller has found the parasite in the healthy human intestine, at times covering large areas of the jejunal surface as a thin membrane. Moritz and Holtz have investigated the frequency of its occurrence and have found it in the evacuations of healthy as well as of diseased individuals, and more frequently in children than in adults. In thirty autopsies made by them, in eight the duodenum contained the parasite. Since it occurred without any lesion of the intestine they believe that it has no pathogenic importance.

IV. Family: Trichomonadidea.

As Trichomonadidea are designated flagellata with a posterior tapering extremity and an anterior end to which are attached several flagella while an undulatory membrane is present along the surface. They have been found in the human vagina, bladder, and intestine. Many of the flagellate parasites found in these organs and described under other names are probably trichomonads, but identification from the descriptions given by their observers is frequently impossible.

Trichomonas Vaginalis, Donné.—The Trichomonas vaginalis was discovered by Donné (1837) in the vaginal secretions of women. The organism is longer than it is broad, measuring 15 to 25 μ in length and 7 to 15 μ in breadth, and one end being rounded, the other pointed, is usually described as having the outline of an apple seed. Attached to the rounded end and arising close together are four flagella which may move so actively that they are distinguished with difficulty. Extending backwards from the point of attachment about half the length of the body is a delicate, very actively motile undulating membrane which has been mistaken for a row of cilia or for a flagellum directed backward. The posterior end is at times prolonged into a tail-like process. An ectosarc and an endosarc are indistinctly distinguishable, the latter containing at times vacuoles and highly refractive granules. The organism is capable of protruding very short pseudopodia. By treatment with acetic acid or by the use of stains a nucleus containing a nucleolus may be demonstrated.

The organism is found with great frequency in the vaginal secretions of both pregnant and non-pregnant women. Kölliker and Scanzoni demonstrated its presence in the vagina of over one-half the women whom they examined during pregnancy and in as large a proportion of those who were not pregnant. Haussmann found the parasite in thirty-seven of two hundred pregnant women and forty times in one hundred women who were not pregnant. These observers have shown that trichomonads occur only in the acid vaginal secretions and frequently in those that contain mucus and leucocytes in considerable quantity; while they found them absent in alkaline secretions containing neither mucus nor leucocytes. It is improbable, however, that the organism is the cause of the inflammatory condition with which it may be associated, since although it frequently occurs in the discharges accompanying gonorrhœa or other conditions in which inflammation of the vaginal tract exists, in many instances of such conditions it is absent.

Trichomonads in the urine have been observed in three instances. Marchand described the first case, that of a man aged sixty years, suffering with a fistula of the perineum. Pus appeared suddenly in the urine, and it was thought possible that rupture had taken place into the bladder. In the urine in association with numerous cocci and bacilli were flagellate organisms, provided with an undulatory membrane and resembling, if not identical with, the Trichomonas vaginalis of Donné. In the freshly passed urine of a man, Miura demonstrated similar organisms and by the introduction of a catheter convinced himself that they came from the urethra and not from the bladder. In the vagina of the patient's wife he was able to demon-

strate the presence of the Trichomonas vaginalis and concluded that he had found the source of the infection. Dock found in the urine of a man suffering with cystitis of obscure etiology trichomonads agreeing in structure with those of the vagina. They were particularly abundant in certain solid particles composed for the most part of desquamated epithelium. Dock was inclined to regard the organism as the cause of the cystitis with which it was associated; in the cases of Marchand and Miura, however, its pathogenicity was by no means established.

It has already been pointed out that there is much obscurity concerning the nature of many of the flagellate organisms found in the intestinal evacuations. The existence of the family Cercomonas, as defined by Grassi, is doubtful, and it is probable that many of the organisms described as cercomonads are in reality trichomonads whose undulatory membrane has been overlooked or erroneously interpreted. The description given by Marchand and by Zunker of organisms found by them in the stools indicate that they studied parasites resembling, if not identical with, the Trichomonas vaginalis, though it is impossible to identify them with any of the species which have become established. Nevertheless, Leuckart, although doubting the complete accuracy of these descriptions, has erected the species Trichomonas intestinalis to include them. In over one hundred cases of diarrhœa Grassi, as previously mentioned, found a flagellate organism to which he gave the name Cercomonas hominis. Its body was pear-shaped and provided with three or four flagella arising from the rounded end, while along one side was an undulating structure which he believed to be a flagellum arising near the others, but directed backward parallel with the surface. In view of subsequent observations it is evident that he described the undulatory membrane characteristic of the trichomonads. In the stools of a patient with carcinoma of the stomach and chronic catarrhal inflammation of the intestine May found a flagellate organism provided with an undulatory membrane and gave it the name Cercomonas coli hominis. Neither May nor Roos, who encountered similar organisms, identify their parasites with the trichomonads. Kruse and Pasquale, however, include in this group similar flagellate parasites which they found associated with diarrhœal conditions whose etiology varied considerably. In the dejections of a typhoid-fever patient and of five patients with enteritis Janowski found trichomonads agreeing in structure with the Trichomonas vaginalis of Donné, and came to the conclusion that the parasite observed by him was identical with the vaginal organism, and with similar bodies found in the urine. Trichomonas hominis he regards as an appropriate name.

What has been said concerning the pathogenic significance of the Megastoma entericum is applicable to the trichomonads of the intestine. Although the parasite is not infrequently found in the diarrhœal discharges of individuals affected with a variety of conditions, for example, typhoid fever, acute and chronic enteritis, there is no evidence that it bears an etiological relation to the pathological process, and, indeed, the variety of the associated conditions is against such a view. Grassi, in studying a large number of cases, found flagellate organisms most frequently in the acute diarrhœas consequent upon indiscretions in diet, and at times was able to demonstrate their presence in great numbers in the fluid evacuations produced by the administration of purgatives. Nevertheless, it is just possible that the parasite when very abundant may, as has been suggested, exacerbate or prolong a diarrhœa already established.

V. Family: Trypanosomata.

The group is composed of organisms provided with an undulatory membrane attached along the body surface and having a single flagellum at one end apparently in continuity with the membrane. The family includes a number of organisms found free in the blood plasma of a variety of vertebrate animals.

The *Herpetomonas lewisii*, Kent, discovered by Osler in the blood of rats is an elongated organism of small size, 1 to 2 μ broad and 25 to 30 μ long, pointed at one end and provided with a flagellum at the other. Along the surface is an undulatory membrane. The *Trypanosoma sanguinis*, of larger size and closely resembling it, is found in the blood of frogs, tortoises, fish, and birds. An undulatory membrane in continuity with a flagellum at one end is spirally attached to the body surface. These organisms are parasitic in apparently healthy animals.

A disease of horses, mules, and camels occurring in India and Burmah is caused by an organism, discovered by Evans, the *Trypanosoma evansi*, resembling closely the trypanosoma of rats. This affection, known as *Surra disease*, is associated with fever and anæmia and is frequently fatal. A similar condition, known as *Nagana* or *Tsetse Fly* disease, affecting horses, asses, cattle, dogs, and other animals, occurs in Africa and is characterized by fever, subcutaneous œdema, emaciation, with moderate anæmia; it runs its course in a few days to several months and is usually fatal. Bruce has found constantly in the blood of animals affected with the disease an organism possessing an undulatory membrane as well as a flagellum. Koch thinks that nagana is identical with surra disease and is caused

by a parasite of identical structure. Kanthack, however, who has recently studied nagana, while confirming and extending the observations of Bruce, does not think that with the insufficient evidence available the two organisms can be identified.

Nagana may be transferred from one animal to another by subcutaneous inoculation with infected blood. Bruce has shown that under ordinary conditions the disease is transmitted by the bite of a blood-sucking insect, the tsetse fly, *Glossina morsitans*, Westwood. He was able to infect healthy animals by causing them to be bitten by tsetse flies obtained from a locality in which the disease was prevalent. According to his observations, the parasite apparently does not undergo development in the body of the insect as does the malarial organism in the mosquito, but, obtained from an infected animal, is transferred to a second individual which is subsequently bitten.

IV. Class: Infusoria.

The Infusoria form a very large group of protozoa whose distinctive feature is the possession of numerous short vibratile processes or cilia arranged in bands or distributed over the entire body.

The members of the group present the greatest diversity and complexity of structure found among the protozoa. The body varies greatly in form and may be ovoid, kidney-shaped, trumpet-shaped, vase-like, etc. Infusoria are not infrequently provided with a stalk by which they may be fixed to some object or to one another, forming aggregations or colonies. The organism is surrounded by a cuticle, perforated by numerous openings for the cilia, which are elongated processes of the cell protoplasm. The arrangement of the cilia upon the surface varies considerably and may be used as a convenient basis of classification. Certain parasitic forms have no mouth for the ingestion of solid food particles, but obtain their nutriment by absorption of material dissolved in the fluid medium about them. In most cases, however, near the anterior end of the body is an opening or mouth frequently surrounded by a special circle of cilia, so arranged as to direct solid particles into it; the mouth is in communication with a short gullet. In many instances at the opposite pole is an anal opening. Within the cell body are usually seen one or two contractile vacuoles.

Infusoria with few exceptions possess two nuclei, each of which is characterized by certain quite constant features. A large oval irregular or elongated body which often stains homogeneously is spoken of as a *megamcleus;* it divides directly by a simple process

of constriction. Almost all species possess a second smaller body, the *micronucleus*, in which division is accomplished by a series of karyokinetic stages. In some species the meganucleus undergoes repeated division so that minute particles of chromatin are scattered diffusely throughout the cell body.

Reproduction by transverse binary fission is almost universal. In certain species spore formation takes place; the organism, becoming encysted, divides into a number of bodies, each of which surrounds itself by a protective covering. In some species conjugation has been observed to precede reproductive division. Of especial interest is a form of conjugation or fertilization occasionally observed in which the micronucleus of two individuals in temporary union divides by karyokinesis into several parts, and one from each organism, passing into the body of the other, fuses with a part which has remained stationary; the essential feature of the process is an interchange of the chromatin material of the micronuclei.

One group of infusoria forming the order Acinetaria is not recognizable in the adult state as ciliate organisms, since cilia are present only at an early stage of development. The full-grown forms are provided with complicated prehensile organs or tentacles. Using the arrangement of cilia as a convenient basis of classification, the remaining members of the class may be grouped into four orders:

I. Holotricha, with cilia over the entire body.

II. Heterotricha, with cilia over the entire body, but with a special set about the mouth stronger than the rest.

III. Hypotricha, whose cilia are limited to the ventral surface.

IV. Peritricha, with cilia arranged in a spiral band about the body.

Of greatest medical interest is the Balantidium coli, a widely distributed parasite belonging to the order Heterotricha. Two similar organisms found like it in the human intestine have been recently described.

Balantidium Coli, Malmsten. *Synonym.*—Paramecium coli.

The Balantidium coli was observed by Malmsten (1857) in the mucous discharges of an individual who, after having passed through an attack of cholera two years before, had subsequently suffered with diarrhœa. It has since been not infrequently found in diarrhœal discharges associated with a variety of intestinal conditions. Its usual seat is the diseased colon, and it occurs abundantly in the mucus discharged. Leuckart has shown that an infusorial organism of identical structure is almost constantly found in the large intestine of pigs, and it is probable that, in many cases at least, the infection is derived from this source. Grassi and Calandruccio, however, were

not able to infect themselves by the ingestion of encysted balantidia from the pig.

The organism is of oval form, slightly more pointed at one end than at the other, and is everywhere covered by cilia which are arranged in parallel rows passing from one to the other pole and giving the surface a striated appearance. An endosarc and an ectosarc are readily distinguishable. Near the anterior or slightly tapering end is a funnel-shaped mouth surrounded by cilia which are larger than those elsewhere; it communicates with a short œsophagus and at the posterior extremity is an anal opening. Within the endosarc is a large kidney-shaped meganucleus and near by a micronucleus. Two contractile vacuoles are usually present.

According to Leuckart, the Balantidium coli of pigs reproduces by transverse fission. In the fæces of this animal occur encysted forms, round bodies provided with a protective capsule.

In the pig the parasite is found in the normal fæces, but in man it occurs in fluid or soft diarrhœal discharges. It is improbable, however, that the organism is the etiological factor in the production of the diarrhœa with which it is associated. Mitter, in twenty-eight cases collected from the literature, found considerable diversity in the concomitant intestinal lesions, among which were those of typhoid fever, tropical dysentery, ankylostoma dysentery, diarrhœa accompanying nephritis, and carcinoma of the rectum—conditions adequate to explain the intestinal disturbance. Moreover, it is not possible to establish a constant relationship between the severity of the diarrhœa and the number of organisms present. The Balantidium coli is apparently an accidental parasite which finds favorable conditions of growth in the diseased intestine.

Jakoby and Schandinn have very recently described two previously unobserved infusoria found in the diarrhœal discharges of a man thirty years of age. In his evacuations were also present both anguillula and ankylostoma eggs. As *Balantidium minutum* they describe an organism whose diameters are about one-third those of the Balantidium coli. Other points of difference are the structure of the mouth, which is a narrow canal extending backward more than one-half the body length, and the arrangement of the cilia. The latter do not form longitudinal rows, but are irregularly distributed upon the surface. More than one contractile vacuole is not present. The Balantidium minutum was found in a second case, observed shortly after its discovery in the first.

Associated with the Balantidium minutum in the case first observed was a second infusorial species to which Jakoby and Schandinn give the name *Nyctotherus faba*. Its body is much smaller than that of

the Balantidium coli; it is flattened from above downwards and has a kidney-shaped outline, being concave along the right margin and convex along the left. The mouth opening is a narrow longitudinal cleft at the right edge and is in communication with a very short œsophagus. Lying in the middle of the body is a round macronucleus whose chromatin is in the form of four or five particles lying upon the inner surface of the nuclear membrane. There is but one contractile vacuole.

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